1: Allergy. 2005 May;60(5):658-64.

Peanut- and cow's milk-specific IgE, Th2 cells and local anaphylactic reaction are induced in Balb/c mice orally sensitized with cholera toxin.

Adel-Patient K, Bernard H, Ah-Leung S, Creminon C, Wal JM.

Laboratoire Inra d'Immuno-Allergie Alimentaire, CEA de Saclay, Gif sur Yvette cedex, France.

BACKGROUND: The development of animal models developing specific immunoglobulin (Ig)E presenting the same specificity as human IgE and similar clinical symptoms as those observed in allergic patients are of great interest for the understanding of mechanisms involved in the induction and regulation of food allergy. METHODS: Balb/c female mice were sensitized with whole peanut protein extract (WPPE) by means of intraperitoneal (i.p.) injections with alum or gavages with cholera toxin (CT). The WPPE specific IgE, IgG1 and IgG2a were monitored. Th2 cells activation was analysed assaying interleukin (IL)-4 and IL-5 vs IFNgamma on reactivated splenocytes. Local anaphylactic reaction was evaluated by assaying histamine in faecal samples. The oral sensitization protocol was further extended to cow's milk proteins (CMP). RESULTS: Balb/c mice developed high peanut-specific IgE and IgG1 responses either after i.p. or oral sensitizations. In both cases, antibodies were specific to polymer of glycinin fragments, containing polypeptides from Ara h3/4, and to a lesser extent to Ara h1 and Ara h2. Interleukin-4 and IL-5 production were evidenced. Balb/c mice could also be sensitized to CMP, as demonstrated by CMP-specific IL-4 and IL-5 secretions and induction of IgE specific for whole caseins, beta-lactoglobulin, serum bovine albumin and lactoferrin. Of interest was the occurrence of a local anaphylactic reaction in the peanut and CM models. CONCLUSIONS: In contrast with previous authors, Balb/c mice were sensitized and evidenced an allergic reaction after oral administrations of peanut or CMP plus CT, providing an interesting model for further studies on immunopathogenic mechanisms.

PMID: 15813812 [PubMed - indexed for MEDLINE]

1: Vaccine. 1994 Oct;12(13):1238-40. Related Articles, Links

Effects of cholera toxin adjuvant on IgE antibody response to orally or nasally administered ovalbumin.

Tamura S, Shoji Y, Hasiguchi K, Aizawa C, Kurata T.

Department of Pathology, National Institute of Health, Tokyo, Japan.

Effects of cholera toxin B subunits supplemented with 0.1% cholera toxin (CTB*) on systemic IgE antibody responses to ovalbumin (OVA) were examined in BDFI (H-2b/d), Balb/c (H-2d) and C3H (H-2k) mice given OVA intragastrically or intranasally. Two successive doses of OVA intragastrically administered to Balb/c and C3H mice induced little IgE response and resulted in almost complete unresponsiveness to subsequent intraperitoneal challenge with OVA in Al(OH)3, while the intragastric administration to BDF1 mice induced high IgE response and resulted in abrogation of the unresponsiveness to the subsequent challenge. The intranasal administration of OVA induced little IgE response and suppressed response to the subsequent challenge in any strain of mice. On the other hand, two successive doses of intragastric or intranasal OVA together with CTB* enhanced IgE response in all three strains and the subsequent challenge with OVA in Al(OH)3 induced higher IgE responses. These results suggest that CTB* augments IgE response to OVA and abrogates the unresponsiveness when administered orally or intranasally into mice together with OVA, regardless of the H-2 haplotype of the mice.

PMID: 7839730 [PubMed - indexed for MEDLINE]

1: Int Arch Allergy Immunol. 2003 Nov;132(3):248-57.

Low-dose oral tolerance due to antigen in the diet suppresses differentially the cholera toxin-adjuvantized IgE, IgA and IgG response.

Christensen HR, Kjaer TM, Frokiaer H.

BioCentrum-DTU, Biochemistry and Nutrition, Technical University of Denmark, Kgs Lyngby, Denmark. hrc@biocentrum.dtu.dk

BACKGROUND: Cholera toxin (CT) is used as a mucosal adjuvant amongst other applications for studying food allergy because oral administration of antigen with CT induces an antigen-specific type 2 response, including IgE and IgA production. Previously established oral tolerance due to antigen in the diet may radically impact on the CT-adjuvantized immune response. The present study served to evaluate the effect of previously established low-dose oral tolerance on the CT-adjuvantized immune response towards a food antigen. METHODS: Mice fed a diet containing microgram levels of the soy protein Kunitz soy-trypsin inhibitor (KSTI) (F0 mice) and mice fed a soy-free diet (F2 mice) were orally immunized with KSTI and CT. KSTI-specific serum IgG1, IgG2a, IgA and IgE and fecal IgA were monitored. KSTI-stimulated cell proliferation and interleukin (IL)-6 production were determined. RESULTS: The anti-KSTI IgE and IgA responses in the F0 mice were substantially suppressed, while the IgG1 and IgG2a responses were not suppressed after five oral immunizations. The response suppression tended to decline with increasing numbers of immunizations suggesting that the suppression could be overcome by multiple immunizations. However, cell proliferation and IL-6 production were clearly suppressed even after five immunizations. CONCLUSIONS: Priorly established low-dose oral tolerance considerably suppressed the CT-adjuvantized KSTI-specific IgE, IgA and cellular immune response but only weakly and transiently the IgG response. The results revealed that low-dose oral tolerance includes the mucosal IgA response and that CT, albeit mediating an antigen-specific response, does not fully abrogate previously established oral tolerance.

1: Curr Drug Targets Inflamm Allergy. 2003 Mar;2(1):31-46.

Erratum in:
- Curr Drug Targets Inflamm Allergy. 2003 Jun;2(2):197.

Immunomodulatory treatment strategies for allergic diseases.

Varga EM, Nouri-Aria K, Till SJ, Durham SR.

Division of Respiratory and Allergic Diseases, Department of Pediatrics, University of Graz, A-8036 Graz, Auenbruggerplatz 30, Austria. evamaria.varga@kfunigraz.ac.at

Over the last decades the prevalence of allergic disorders, such as hayfever and asthma has increased worldwide, mostly in westernised countries where up to 20 % of the population are affected. The "hygiene hypothesis" suggests that modernised lifestyles such as improved housing conditions, altered dietary habits and smaller family sizes may be responsible for the decrease in infectious and the increase in allergic diseases. Childhood atopic diseases, like eczema, food allergies and recurrent wheezy bronchitis represent a considerable health problem and a major socioeconomic burden due to the chronicity of these disorders. In recent years, a better understanding of the immunopathogenesis of allergic diseases has evolved, which has contributed to the development of novel more targeted forms of therapy. Allergen injection immunotherapy is the only treatment in current use with the potential for modifying the course of allergic disease. In order to better target mucosal allergies, new approaches of administering allergen, via the sublingual or intranasal route, are being developed. The use of modified allergens, allergen peptides, DNA immunization and the use of novel adjuvants represent alternatives to conventional immunotherapy with potential for improved efficacy with less side effects. For atopic asthma, novel treatment strategies aim at locally targeting inflamed airways. Nebulized monoclonal blocking antibodies and soluble interleukin receptors against "Th(2)-type" cytokines have been designed. An alternative approach has been the administration of "Th(1) -type" cytokines. Although, immunomodulatory strategies provide a promising outlook for the treatment of allergic patients, more studies are needed in the future to address issues of

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☐	L25	L24 and allergy	106
☐	L24	L23 and treatment	224
☐	L23	(enterotoxin)same(E)adj(coli)same(B)adj(subunit)	261
☐	L22	L21 and (B)adj(subunit)	49
☐	L21	L16 and enterotoxin	110
☐	L20	L16 and EtxB	0
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☐	L15	L14 and eczema	2056
☐	L14	L1 and rhinitis	4496
☐	L13	5980898.pn.	2
☐	L12	5930106.pn.	2
☐	L11	(cholera)adj(toxin)adj(B)adj(subunit)same(allergy)	5
☐	L10	(CtxB)same(allergy)	0
☐	L9	(EtxB)same(allergy)	2
☐	L8	L7 and (cholera)adj(toxin)same(B)adj(subunit)	118
☐	L7	L1 and toxin	2694
☐	L6	L3 and rhinitis	17
☐	L5	L4 and asthma	19
☐	L4	L3 and (B)adj(subunit)	53
☐	L3	L2 and (cholera)adj(toxin)	106
☐	L2	L1 and (mucosal)adj(adjuvant)	118
☐	L1	(allergy)same(treatment)	14731

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L3   ANSWER 1 OF 2  CAPLUS  COPYRIGHT 2006 ACS on STN
2006:157389  Document No. 144:285812  Effect of antiallergic agents on
     scratching behavior in magnesium and zinc deficiency hairless mice.
     Makiura, Munehiko; Akamatsu, Hirohiko; Yagami, Akiko; Shimizu, Yoshinori;
     Matsunaga, Kayoko (Dep. Dermatol., Sch. Med., Fujita Health University,
     Toyoake, 470-1192, Japan).  Nippon Hifuka Gakkai Zasshi, 115(13),
     2228-2231 (Japanese) 2005.  CODEN: NHKZAD.  ISSN: 0021-499X.  Publisher:
     Nippon Hifuka Gakkai.
AB   Groups of 6 HR-1 hairless mice were fed with a conventional diet (CD) or a
     special diet (SD) 50% lower in Mg and Zn contents than those of the CD for
     5 wk from 4-wk-old.  Mice fed with the SD were orally given fexofenadine
     HCl (FEX) at 1.67 or 3.34 mg/kg/day or cetirizine HCl (CET) at 20 or 40
     mg/kg/day during the feeding period.  Frequency of scratching behavior
     (SB) was counted for 30 min weekly from 3 to 5 wk after starting
     drug-administration.  SB-frequency in each measurement was significantly
     higher in the SD alone group than in the CD alone group.  SB-frequency was
     significantly decreased in the high dose FEX-treated group.  No
     significant alteration in SB-frequency was observed in the low dose
     FEX-treated group and the high and low dose CET-treated groups.  Epidermal
     thickening and inflammatory cell infiltration in dermis were stronger in
     the SD alone group than in the CD alone group.  In the high dose FEX- and
     CET-treated groups, these histopathol. findings attenuated.  Nos. of skin
     mast cells (MCs) and eotaxin (ETX)-pos. cells (EPCs)
     significantly increased in the SD alone group as compared with the CD
     alone group.  High dose FEX significantly decreased both MCs and EPCs nos.
     and high dose CET significantly decreased EPCs number only.  Plasma histamine
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(H) and **ETX** concns. were significantly higher in the SD alone group than in the CD alone group. High dose FEX significantly decreased both H and **ETX** concns. and high dose CET significantly decreased **ETX** concentration only. These results indicate that the pharmacol. activity of FEX is stronger than that of CET and the difference is probably due to the difference of drug absorption.

L3 ANSWER 2 OF 2 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN
2004:328080 Document No.: PREV200400325706. Presence of staphylococcal exfoliative toxin A in sera of patients with atopic dermatitis. Yagi, S. [Reprint Author]; Wakaki, N.; Ikeda, N.; Takagi, Y.; Uchida, H.; Kato, Y.; Minamino, M.. Div Res and DevInst Cosmet Sci, Club Cosmet Co Ltd, 145-1 Ichibu Cho, Ikoma, Nara, 6300222, Japan. syagi@clubcosmetics.co.jp. Clinical and Experimental Allergy, (June 2004) Vol. 34, No. 6, pp. 984-993. print.
ISSN: 0954-7894 (ISSN print). Language: English.

AB Background It has been reported that the toxins that Staphylococcus aureus produces are associated with the exacerbation of atopic dermatitis (AD). It has been shown in many studies that staphylococcal enterotoxin (SE) A and SEB contribute to AD by humoral immunity through IgE production as a superantigen. On the other hand, little attention has been paid to the relationship between AD and exfoliative toxin x (**ETx**). Objective We investigated the toxins that are frequently detected from the skin of patients and how these toxins affect AD. Methods S. aureus, isolated from the skin of 100 patients with mild to severe AD, were examined for the producibility of toxins by polymerase chain reaction. Serum samples were obtained from 21 patients with mild and moderate AD. The levels of SEB, ETA, total IgE, specific IgE, and specific IgG in sera were measured by ELISA. Results SEB was most frequently detected from S. aureus on the skin of these patients as previously reported. And **ETx**, to which little attention has been paid so far, was frequently detected next to SEB. Furthermore, ETA was detected from the sera of almost all the AD patients. SEB was not detected at all. Although the level of ETA in the AD group was significantly higher than that of controls, ETA-specific IgE was not detected from their sera. High levels of ETA tended to be detected from infantile patients. Although there were no significant differences in the levels of ETA-IgG between AD and the controls, its prevalence was more than twice as high as the controls in AD. Conclusion These results suggest that many AD patients were exposed to **ETx**. We conclude that **ETx** may contribute to exacerbation of AD, particularly in infants, by a mechanism that is not through specific IgE production, unlike SEB.

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L5 ANSWER 1 OF 13 CAPLUS COPYRIGHT 2006 ACS on STN
2005:99518 Document No. 142:204523 Agonist peptides of PAR-2, a human receptor for zonulin and for Vibrio phage **CTX**.phi. ZOT, and uses to facilitate drug and antigen absorption, and in therpay and diagnosis. Fasano, Alessio; Vogel, Stefanie N. (University of Maryland, Baltimore, USA). PCT Int. Appl. WO 2005010022 A2 20050203, 55 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH,

PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2004-US22753 20040715. PRIORITY: US 2003-2003/PV487889 20030715.

AB The invention provides an agonist polypeptide of a human receptor of zonulin and Vibrio cholerae phage **CTX**.phi. ZOT (zonula occludens toxin) protein. The agonist can be used to facilitate drug and antigen absorption. Suitable routes of administration include oral, nasal, transdermal, and i.v. Pharmaceutical formulations may comprise a therapeutic agent or an immunogenic agent in combination with the agonist polypeptide. It was shown, that proteinase-activated receptor PAR-2 variant or homolog is the target receptor for both Zot and zonulin, and suggested that this receptor is involved in the regulation of intercellular tight junction. It was also shown, that Zot binds directly to the PAR-2 ECL2 and activates the receptor signaling, while zonulin may activate the target receptor by cleaving it at its N-terminus.

L5 ANSWER 2 OF 13 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN

2003:357927 Document No.: PREV200300357927. Antibody to Ceftriaxone in HIV Pediatric Patients and Potential Implications for RBC Hemolysis. Bateman, Scot T. [Reprint Author]; Hu, Edward [Reprint Author]; Lane, Cathy [Reprint Author]; Quillen, Karen [Reprint Author]; Pelton, Stephen I. [Reprint Author]. Pediatrics, Boston University School of Medicine, Boston, MA, USA. Blood, (November 16 2002) Vol. 100, No. 11, pp. Abstract No. 3656. print.
Meeting Info.: 44th Annual Meeting of the American Society of Hematology. Philadelphia, PA, USA. December 06-10, 2002. American Society of Hematology.
CODEN: BLOOAW. ISSN: 0006-4971. Language: English.

AB Introduction: Ceftriaxone (**CTX**), a third generation cephalosporin, is commonly used for the treatment of suspected bacteremia in high risk patients because of its broad spectrum antibacterial activity and prolonged half-live. Significant complications of severe immune hemolytic anemia (IHA) secondary to parenteral **CTX** have recently been reported. Of the cases reported, 6/9 were in children and 5/6 were fatal. All of the patients were immunocompromised or had underlying chronic hematologic disorders - HIV infection, sickle cell disease, hypereosinophilic syndrome, or leukemia and had a history of receiving multiple courses of **CTX**. **CTX** appears unique in that all reported cases of ceftriaxone associated hemolysis have reacted only by the immune complex mechanism. This mechanism appears to produce the most fulminant clinical picture. The prevalence of **CTX**-induced drug-dependent RBC antibodies in an at-risk population has not previously been studied. Methods: All patients (age 1 yr to 21 yr) followed in the Pediatric HIV clinic were eligible to participate. IRB approval was obtained. Serum from clinical specimens after all tests for the patient's care were completed was used. The patient's serum was incubated in the presence of **CTX**, with and without the addition of fresh normal serum as a source of complement, with untreated and enyzme-treated donor RBCs. If enough serum was available, the testing was repeated with cefuroxime, another third generation cephalophorin, which has no IHA case reports . Positive agglutination or hemolysis in any of the patient's tests to which the drug was added, and a negative or significantly weaker result in the corresponding negative control tests suggested the presence of an antibody to the drug being studied. Additionally, we correlated our results with **CTX** exposure history on review of pt medical record. Positive controls (sera known to contain **CTX**-induced RBC antibodies) and other technical assistance was obtained from the Immunohematology Research Laboratory at the American Red Cross Blood Services, Southern California Region. Results: 29 pediatric HIV pt were screened. Mean age was 10.8 yr (range of 3 to 19yr), mean number of treatment courses (q day dosing x 1-2 days up to 3wk/course) of

CTX was 4 (+-3.8, range 0-20). Overall incidence of a positive (+) **CTX** dependent RBC antibody test was 14% (4/29). Another 24% (7/29) had non-interpretable (NI) results secondary to the presence of allo-or auto-antibodies. All three groups of pt were similar in respect to age (11.2 (+) vs. 11.4 (-) vs. 10.4 (NI)) as well as known drug **allergies**, baseline medications and known complications. Positive antibody patients had less exposure to **CTX** than (-) patients 2.3 vs. 5.1 courses, and similar to NI patients 2.1 courses. Thirteen patients were tested with cefuroxime (including 3/4 who tested + to **CTX**). 1/13 was positive (and also positive to **CTX**), 2/13 were NI (and also NI to **CTX**), and 10/13 negative (including 2/3 who were + to **CTX**). Conclusions: A significant number of pediatric HIV patients with repeated exposure to **CTX** appear to have the potential for IHA but the mechanism for its development remains unclear. A more thorough prospective investigation into the development and risk potential is warranted prior to making recommendations about drug therapy.

L5 ANSWER 3 OF 13 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN
1996:145016 Document No.: PREV199698717151. Interferon gamma (IFN-gamma) inhibits neutrophil chemotaxis (**CTX**) without affecting generation of superoxide anion (SO). Kowalski, M. L.; Szkudlinska, B.; Pawliczak, R.; Iwaszkiewicz, J.. Lodz, Poland. Journal of Allergy and Clinical Immunology, (1996) Vol. 97, No. 1 PART 3, pp. 282.
Meeting Info.: Fifty-second Annual Meeting of the American Academy of Allergy Asthma and Immunology. New Orleans, Louisiana, USA. March 15-20, 1996.
CODEN: JACIBY. ISSN: 0091-6749. Language: English.

L5 ANSWER 4 OF 13 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN
1996:356096 Document No.: PREV199699078452. Inhibition of neutrophil chemotaxis (**CTX**) by recombinant interferon gamma (rINF-gamma). Kowalski, M. L.; Szkudlinska, B.; Pawliczak, R.; Iwaszkiewicz, J.; Woszczek, G.. Lodz, Poland. Allergy (Copenhagen), (1996) Vol. 51, No. SUPPL. 31, pp. 33.
Meeting Info.: Annual Meeting of the European Academy of Allergology and Clinical Immunology. Budapest, Hungary. June 2-5, 1996.
CODEN: LLRGDY. ISSN: 0105-4538. Language: English.

L5 ANSWER 5 OF 13 CAPLUS COPYRIGHT 2006 ACS on STN
1996:266150 Document No. 124:332301 Immunomodulating effects of the extract from clam Meretrix meretrix on delayed hypersensitivity in mice. He, Yajun; Wu, Qian; Zhu, Reifei (Guangdong Province Institute Materia Medica, Canton, 510180, Peop. Rep. China). Zhongguo Haiyang Yaowu, 14(3), 020-1 (Chinese) 1995. CODEN: ZHYAE8. ISSN: 1002-3461. Publisher: Shandong Haiyang Yaowu Kexue Yanjiuso.
AB The immunomodulating effects of the extract from Meretrix meretrix on the delayed hypersensitivity (DH) in mice were studied. The results showed that the extract from Meretrix meretrix and its polysaccharides promoted the DH-decreasing effect of cyclophosphamide (**CTX**), and inhibited the DH-increasing effect of **CTX**.

L5 ANSWER 6 OF 13 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN
DUPLICATE 1
1994:246123 Document No.: PREV199497259123. Cholera toxin (**CTX**) promotes IgE antibody and **allergy** during oral immunization. Snider, D. P.; Marshall, J. S.; Perdue, M. H.; Liang, H.. Dep. Pathology, MVIP, McMaster Univ., Hamilton, ON L8N 3Z5, Canada. FASEB Journal, (1994) Vol. 8, No. 4-5, pp. A282.
Meeting Info.: Experimental Biology 94, Parts I and II. Anaheim, California, USA. April 24-28, 1994.
CODEN: FAJOEC. ISSN: 0892-6638. Language: English.

L5 ANSWER 7 OF 13 CAPLUS COPYRIGHT 2006 ACS on STN

1991:239997 Document No. 114:239997 Antigenicity study of cefpirome sulfate. Inoue, Sachiko; Morioka, Hiroshi; Satoh, Ryoichi; Yoshida, Yasushi; Omosu, Mikio; Kobayashi, Takayoshi (Pharma Res. Lab., Hoechst Japan Ltd., Kawagoe, 350, Japan). Journal of Toxicological Sciences, 15(Suppl. 3), 129-45 (Japanese) 1990. CODEN: JTSCDR. ISSN: 0388-1350.

AB Immunol. properties of cefpirome sulfate (CPR) were examined The immunogenicity and challenging ability of CPR were examined in guinea pigs by active systemic anaphylaxis (ASA) and homologous 4-h passive cutaneous anaphylaxis (PCA) tests. The animals given CPR alone i.p. for sensitization and their sera were neg. for ASA or PCA reactions, like the results with reference substances, ceftazidime (CAZ) and cephalothin sodium (CET). When each antibiotic plus Freund's complete adjuvant (FCA) was used for sensitization, ASA reactions were observed with CPR, cephaloridine (CER), CET, and cefazolin sodium (CEZ), and PCA reactions, with CPR and CET. CPR had the ability to challenge the ASA and PCA reactions. CER and CET also showed the ability to challenge ASA or PCA reactions, though at low incidences. The cross-reactivity of CPR with com. available antibiotics was examined by heterologous PCA test and by passing hemagglutination test and its inhibition test. The antiserum used was from rabbits immunized with each antibiotic-ovalbumin conjugate plus FCA, and the antigen was each antibiotic-bovine serum albumin conjugate. CPR cross-reacted markedly with cefotaxime sodium (**CTX**) having the same side chain at position 7 and showed weak, unidirectional reactions with CAZ and CET. In the in vitro direct Coombs test, the pos. reactions noted with CPR were stronger than those with latamoxef sodium, equal to those with CEZ and slighter than those with **CTX**, CET and benzylpenicillin potassium. In conclusion, in the safety evaluation of CPR, its antigenic potential may not be a problem, like the cases of other antibiotics.

L5 ANSWER 8 OF 13 CAPLUS COPYRIGHT 2006 ACS on STN

1984:628385 Document No. 101:228385 Characterization of the delayed hypersensitivity response to a protein antigen in the mouse - I. Kinetics of reactivity and sensitivity to classical immunosuppressants. Holsapple, Michael P.; Page, Dennis G.; Bick, Peter H.; Shopp, George M. (Med. Coll. Virginia, Virginia Commonw. Univ., Richmond, VA, 23298, USA). International Journal of Immunopharmacology, 6(5), 399-405 (English) 1984. CODEN: IJIMDS. ISSN: 0192-0561.

AB Several parameters of the delayed hypersensitivity response (DHR) to a protein antigen, keyhole limpet hemocyanin (KLH), were investigated. Female B6C3F1 mice were sensitized with KLH suspended in either complete Freund's Adjuvant (CFA) or sterile saline. When the mice were sensitized twice, the magnitudes of these responses were equivalent as measured by a radioisotope procedure reflecting the influx of monocytes. With only a single sensitization, there was a 37% decrease in the response of CFA-treated mice and a dramatic (82%) decrease in the response of saline-treated mice. Utilizing 2 sensitizing injections in male CD-1 mice, the kinetics of the responses were determined to be equivalent regardless of whether KLH was suspended in CFA or saline in that both responses were persistent for up to 5 wks between the second sensitization and challenge. Ear thickness in CFA-treated mice was twice that of the saline-treated mice at 1, 3 and 5 wks. This increased swelling was not due to an increase in the vascular permeability as measured by the extravasation of a radiolabeled protein. There was a marked increase in the total area of fibrin in both sensitized groups when compared to unsensitized mice, but no difference between the groups. The sensitivity of these responses to immunosuppressants was determined in male CD-1 mice exposed subchronically (14 day) to dexamethasone (DEX) and cytoxan (**CTX**). There was a marked increase in the suppression by DEX in mice sensitized to KLH in saline as compared to mice sensitized to KLH in CFA. In contrast, the sensitivity to suppression by cyclophosphamide was not affected by the

presence of CFA.

L5 ANSWER 9 OF 13 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN
1980:141815 Document No.: PREV198069016811; BA69:16811. DELAYED HYPER SENSITIVITY IN SURGICAL PATIENTS A MECHANISM FOR ANERGY. CHRISTOU N V [Reprint author]; MEAKINS J L. DEP SURG, R VICTORIA HOSP, ROOM S624, 687 PINE AVE W, MONTREAL, QUE 13A 1A1, CAN. Surgery (St Louis), (1979) Vol. 86, No. 1, pp. 78-85.
CODEN: SURGAZ. ISSN: 0039-6060. Language: ENGLISH.
AB Lymphocyte chemotaxis (**CTX**) was studied in 74 patients and 13 controls to assess whether or not failed delayed hypersensitivity (DH) skin test response might be due to a failure of recruitment of lymphocytes to the area of antigen deposition. Lymphocytes were obtained by Ficoll-Hypaque separation of leukocyte-rich plasma and used at a concentration of 3 + 106 cells/ml in minimal essential medium plus 10% fetal calf serum (MEM-FCS). **CTX** was assessed using 3 μm nitrocellulose filters, casein (5 mg/ml) in MEM-FCS as attractant, incubation at 37° C for 5 h and the leading front technique. In this system control lymphocytes migrated a distance of 106.4 ± 1.6 μm (n = 13, mean ± SE), whereas lymphocytes from anergic patients migrated 87.5 ± 1.3 μm (n = 35, P < 0.0005). Simultaneous determinations of lymphocyte **CTX** and PMN [polymorphonuclear leukocyte] **CTX** demonstrated that the 2 are highly correlated (X = 22.4 ± 0.67 Y, r2 = 0.7904, P < 0.0005). Anergic sera which decreased control PMN **CTX** from 128.1 ± 2.4 to 94.7 ± 1.5 μm also decreased control lymphocyte **CTX** from 112.1 ± 3.1 to 81.5 ± 2.4 μm (n = 13, P < 0.001). Stimulation index following blastogenic transformation of lymphocytes with phytohemagglutinin was similar for anergic patients (11.5) compared to controls (13) (P .apprx. 0.5). The failure of DH responses seen in surgical patients probably does not reflect classical cell-mediated immunity. This newly demonstrated defect in lymphocyte **CTX** in anergic patients, together with reduced neutrophil **CTX** (mediated by circulating serum inhibitors), apparently contributes to the observed failed DH response.

L5 ANSWER 10 OF 13 CAPLUS COPYRIGHT 2006 ACS on STN
1978:458241 Document No. 89:58241 The role of B lymphocytes in cell-mediated immunity. II. Delayed hypersensitivity induced by dinitrophenyl-Ficoll in dinitrophenyl-keyhole limpet hemocyanin-immunized guinea pigs. Rosenstreich, David L.; Wahl, Sharon M.; McMaster, Philip R. B. (Natl. Inst. Dent. Res., NIH, Bethesda, MD, USA). Cellular Immunology, 38(1), 116-23 (English) 1978. CODEN: CLIMB8. ISSN: 0008-8749.
AB Dinitrophenyl (DNP)-Ficoll will elicit typical delayed hypersensitivity skin reactions in guinea pigs immunized with DNP-keyhole limpet hemocyanin (KLH). Lymph node cells (LNC) from these animals produced the lymphokine, monocyte chemotactic factor (MNL **CTX**) when stimulated by DNP-Ficoll in vitro. This response was antigen and hapten specific since LNC from nonimmune guinea pigs or those immunized with nonDNP containing antigens were not stimulated by DNP-Ficoll. Lymph node cells were fractionated into T- and B-cell-enriched populations to determine the nature of the DNP-Ficoll-responsive cell. Only the B-lymphocyte-enriched population produced MNL **CTX** in response to DNP-Ficoll. The purity of the B-cell population was demonstrated by its failure to respond to PHA and by the fact that B cells derived from DNP-ovalbumin (OVA) immune guinea pigs responded to both lipopolysaccharide and to DNP-Ficoll, although they could no longer respond without T-cell help to the T-dependent antigen, DNP-OVA. These findings suggest that the hapten-specific response to guinea pigs to DNP-Ficoll may be a form of B-cell-mediated delayed hypersensitivity.

L5 ANSWER 11 OF 13 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN
1978:165899 Document No.: PREV197865052899; BA65:52899. DELAYED HYPER

SENSITIVITY INDICATOR OF ACQUIRED FAILURE OF HOST DEFENSES IN SEPSIS AND TRAUMA. MEAKINS J L [Reprint author]; PIETSCH J B; BUBENIK O; KELLY R; RODE H; GORDON J; MACLEAN L D. ROOM S1030, 687 PINE AVE W, MONTREAL, QUE H3A 1A1, CAN. Annals of Surgery, (1977) Vol. 186, No. 3, pp. 241-250. CODEN: ANSUA5. ISSN: 0003-4932. Language: ENGLISH.

AB Primary failure of host defense mechanisms has been associated with increased infection and mortality. Anergy, the failure of delayed hypersensitivity response, identifies surgical patients at increased risk for sepsis and related mortality. The anergic and relatively anergic patients whose skin tests failed to improve had a mortality rate of 74.4%, whereas those who improved their responses had a mortality rate of 5.1% ($P < 0.001$). This study documents abnormalities of neutrophil chemotaxis, T [thymus-derived] lymphocyte rosetting in anergic patients and the effect of autologous serum. These abnormalities may account for the increased infection and mortality rates in anergic patients. Skin testing with 5 standard antigens identified 110 anergic (A) or relatively anergic (RA) patients in whom neutrophil chemotaxis (**CTX**) and bactericidal function (NBF), T lymphocyte rosettes, mixed lymphocyte culture (MLC), cell-mediated lympholysis (CML) and blastogenic factor (BF) were studied. The MLC, CML and BF were normal in the patients studied and were not clinically helpful. Neutrophil **CTX** in 19 controls was 117.5 ± 1.6 u [units], whereas in 40 A patients, neutrophils migrated 81.7 ± 2.3 u, and in 15 RA patients 97.2 ± 3.8 u ($P < 0.01$). In 14 patients whose skin tests converted to normal, neutrophil migration improved from 78.2 ± 5.4 u to 107.2 ± 4.0 u ($P < 0.01$). Incubation of A or control neutrophils in A serum reduced migration in A patients from 93 ± 3.7 u to 86.2 ± 3.5 u ($P < 0.01$) and in normals from 121.2 ± 1.6 u to 103.6 ± 2.6 u ($P < 0.001$). The percent rosette forming cells in 66 A patients was 42.5 ± 3.1 compared to 53.6 ± 2.8 in normal responders ($P < 0.02$). Incubation of normal lymphocytes in anergic serum further reduced rosetting by 30%. Restoration of delayed hypersensitivity responses and concurrent improvement in cellular and serum components of host defense were correlated with maintenance of adequate nutrition and aggressive surgical drainage.

L5 ANSWER 12 OF 13 CAPLUS COPYRIGHT 2006 ACS on STN

1975:71504 Document No. 82:71504 Characterization of chemotactic activity produced in vivo by a cell mediated immune reaction in the guinea pig. Postlethwaite, Arnold E.; Snyderman, Ralph (Lab. Connect. Tissue Res., VA Hosp., Memphis, TN, USA). Journal of Immunology, 114(1), 275-9 (English) 1975. CODEN: JOIMA3. ISSN: 0022-1767.

AB The mechanisms of leukocyte accumulation in vivo were examined in guinea pigs exhibiting delayed hypersensitivity to horseradish peroxidase (HRPO). Within 24 hr of the i.p. injection of HRPO to such animals there was a significant increase in the number of peritoneal macrophages and in the chemotactic activity (**CTX**) for macrophages in the sampled peritoneal fluid. At 48 and 72 hr the **CTX** returned to the prechallenge level and i.p. macrophages appeared to be actively phagocytic. Mol. sieve chromatograms of concentrated peritoneal fluid obtained 24 hr after i.p. challenge with HRPO and of supernatants derived from immune spleen cells cultured in the presence of HRPO in vitro revealed that the major portion of **CTX** for homologous macrophages eluted in the region of the 12,500 dalton protein marker. The partially purified **CTX** obtained from peritoneal fluid and supernatants of spleen cell cultures was heat stable (56° for 30 min) and was destroyed by trypsin digestion. Thus, a chemotactic factor for macrophages, similar to a lymphocyte-derived chemotactic factor obtained in vitro, is present in vivo at the site of a cell-mediated immune reaction.

L5 ANSWER 13 OF 13 CAPLUS COPYRIGHT 2006 ACS on STN

1974:424087 Document No. 81:24087 In vitro studies of a chemotactic lymphokine in the guinea pig. Wahl, Sharon M.; Altman, Leonard C.; Oppenheim, Joost J.; Mergenhagen, Stephan E. (Natl. Inst. Dent. Res.,

Natl. Inst. Health, Bethesda, MD, USA). International Archives of Allergy and Applied Immunology, 46(5), 768-84 (English) 1974. CODEN: IAAAAM. ISSN: 0020-5915.

AB Lymphocytes from guinea pigs with delayed cutaneous reactivity to dinitrophenyl ovalbumin (DNP-OA) when challenged with this antigen in vitro elaborated a small mol. weight mediator which is chemotactic for monocytes. The in vitro production of mononuclear leukocyte (MNL) chemotactic factor (**CTX**) was carrier specific and correlated with delayed hypersensitivity. Production of this lymphokine by specific antigen or mitogen-stimulated spleen cells occurred within 8-24 hr of incubation, preceding measurable lymphocyte proliferation. MNL **CTX** was sensitive to proteases from C5 and other guinea pig lymphokines.

=> s l1 and EtxB
L6 1 L1 AND ETXB

=> d l6 cbib abs

L6 ANSWER 1 OF 1 CAPLUS COPYRIGHT 2006 ACS on STN
1999:451202 Document No. 131:82960 **EtxB** or ganglioside GM1 for treating allergic or hypersensitivity conditions. Williams, Neil Andrew; Hirst, Timothy Raymond; Bienenstock, John (Oratol Limited, UK). PCT Int. Appl. WO 9934817 A1 19990715, 46 pp. DESIGNATED STATES: W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1999-GB70 19990108. PRIORITY: GB 1998-487 19980109.

AB The use of an agent in the manufacture of a medicament to treat an allergic condition and/or a hypersensitivity condition is described. The agent is capable of modulating a ganglioside-associated activity. The agent is not coupled to an antigen. The modulation of the ganglioside-associated activity affects an allergic condition and/or a hypersensitivity condition. Examples of such modulators include ganglioside GM1 and E. coli enterotoxin B subunit.

=> s l1 and CtxB
L7 1 L1 AND CTXB

=> d l7 cbib abs

L7 ANSWER 1 OF 1 CAPLUS COPYRIGHT 2006 ACS on STN
2000:814337 Document No. 133:361908 Bacteriophage isolated from bacterial genomes and extrachromosomal elements and methods of use thereof. Karaolis, David K. R. (University of Maryland, Baltimore, USA). PCT Int. Appl. WO 2000067784 A1 20001116, 59 pp. DESIGNATED STATES: W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 2000-US12580 20000510. PRIORITY: US 1999-PV133373 19990510.

AB The present invention relates to compns., methods, processes, etc., relating to bacteriophage which are encoded by chromosome, plasmids, or an extrachromosomal element of bacteria. The bacteriophage of the present

invention are preferably encoded by pathogenicity islands in chromosomes or plasmids of pathogenic bacteria. The bacteriophage can be utilized as a pharmaceutical composition, e.g., to elicit an immune response, e.g., for the purpose of producing antibodies, as vaccines and vaccine vectors to regulate the immune system, e.g., for the prevention and treatment of **allergy**, disease, and other pathol. conditions. The invention finds addnl. utility in systems and methods for the detection of pathogens comprising bacteriophage and a system and method for the environmental eradication of pathogenic microorganisms.

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=> s enterotoxin B
L8        9729 ENTEROTOXIN B

=> s l8 and allergen
L9         141 L8 AND ALLERGEN

=> dup remove l9
PROCESSING COMPLETED FOR L9
L10          60 DUP REMOVE L9 (81 DUPLICATES REMOVED)

=> s l10 and treatment
L11          12 L10 AND TREATMENT

=> dup remove l11
PROCESSING COMPLETED FOR L11
L12          12 DUP REMOVE L11 (0 DUPLICATES REMOVED)

=> d l12 1-12 cbib abs

L12  ANSWER 1 OF 12     MEDLINE on STN
2005657544.    PubMed ID: 16339573.   Topical superantigen exposure induces
     epidermal accumulation of CD8+ T cells, a mixed Th1/Th2-type dermatitis
     and vigorous production of IgE antibodies in the murine model of atopic
     dermatitis. Savinko Terhi; Lauerma Antti; Lehtimaki Sari; Gombert Michael;
     Majuri Marja-Leena; Fyhrquist-Vanni Nanna; Dieu-Nosjean Marie-Caroline;
     Kemeny Lajos; Wolff Henrik; Homey Bernhard; Alenius Harri. (Laboratory of
     Immunotoxicology, Finnish Institute of Occupational Health, Helsinki,
     Finland. ) Journal of immunology (Baltimore, Md. : 1950), (2005 Dec 15)
     Vol. 175, No. 12, pp. 8320-6.  Journal code: 2985117R. ISSN: 0022-1767.
     Pub. country: United States. Language: English.
AB   Patients with atopic dermatitis (AD) have repeated cutaneous exposure to
     both environmental allergens and superantigen-producing strains
     of Staphylococcus aureus. We used a murine model of AD to investigate the
     role of staphylococcal enterotoxin B (SEB) in the
     modulation of allergen-induced skin inflammation. Mice were
     topically exposed to SEB, OVA, a combination of OVA and SEB (OVA/SEB), or
     PBS. Topical SEB and OVA/SEB exposure induced epidermal accumulation of
     CD8+ T cells and TCRVbeta8+ cells in contrast to OVA application, which
     induced a mainly dermal infiltration of CD4+ cells. SEB and OVA/SEB
     exposure elicited a mixed Th1/Th2-associated cytokine and chemokine
     expression profile within the skin. Restimulation of lymph node cells
     from OVA- and OVA/SEB-exposed mice with OVA elicited strong production of
     IL-13 protein, whereas substantial amounts of IFN-gamma protein were
     detected after SEB stimulation of cells derived from SEB- or
     OVA/SEB-exposed mice. Topical SEB treatment elicited vigorous
     production of SEB-specific IgE and IgG2a Abs and significantly increased
     the production of OVA-specific IgE and IgG2a Absolute The present study shows
     that topical exposure to SEB provokes epidermal accumulation of CD8+ T
     cells, a mixed Th2/Th1 type dermatitis and vigorous production of specific
     IgE and IgG2a Abs, which can be related to the chronic phase of atopic
     skin inflammation.
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L12 ANSWER 2 OF 12 MEDLINE on STN

2006007409. PubMed ID: 16393321. Clinical effects of probiotics are associated with increased interferon-gamma responses in very young children with atopic dermatitis. Prescott S L; Dunstan J A; Hale J; Breckler L; Lehmann H; Weston S; Richmond P. (School of Paediatrics and Child Health, University of Western Australia, Perth, WA, Australia.. susanp@ichr.uwa.edu.au) . Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology, (2005 Dec) Vol. 35, No. 12, pp. 1557-64. Journal code: 8906443. ISSN: 0954-7894. Pub. country: England: United Kingdom. Language: English.

AB BACKGROUND: We recently demonstrated that administration of probiotics resulted in significant clinical improvement in very young children with moderate-to-severe atopic dermatitis (AD). The purpose of this study was to determine the underlying immunological effects that are associated with these apparent clinical benefits. METHODS: Peripheral blood mononuclear cells (PBMC) were isolated from children (n = 53) at baseline and at the end of an 8-week supplementation period during which they received a probiotic (Lactobacillus fermentum PCCtrade mark) (n = 26) or a placebo (n = 27). A further sample was collected at 16 weeks (8 weeks after ceasing the supplement). Cytokine (IL-5, IL-6, IL-10, IL-13, IFN-gamma and TNF-alpha) responses to **allergens** (egg ovalbumin (OVA), beta lactoglobulin (BLG), house dust mite (HDM)), vaccines (tetanus toxoid (TT)), diphtheria toxoid (DT)), intestinal flora (heat-killed Lactobacillus (HKLB)), heat-killed Staphylococcus aureus (HKSA), Staphylococcus aureus **enterotoxin B** (SEB) and mitogen (phytohaemaglutinin (PHA)) were compared. RESULTS: The administration of probiotics was associated with a significant increase in T-helper type 1(Th1-type) cytokine IFN-gamma responses to PHA and SEB at the end of the supplementation period (week 8: P = 0.004 and 0.046) as well as 8 weeks after ceasing supplementation (week 16: P = 0.005 and 0.021) relative to baseline levels of response. No significant changes were seen in the placebo group. The increase in IFN-gamma responses to SEB was directly proportional to the decrease in the severity of AD (r = -0.445, P = 0.026) over the intervention period. At the end of the supplementation period (week 8) children receiving probiotics showed significantly higher TNF-alpha responses to HKLB (P = 0.018) and HKSA (P = 0.011) but this was no longer evident when supplementation ceased (week 16). Although IL-13 responses to OVA were significantly reduced in children receiving probiotics after 8 weeks (P = 0.008), there were no other effects on **allergen**-specific responses, and this effect was not sustained after ceasing supplementation (week 16). There were no effects on vaccine-specific responses, or on responses to any of the stimuli assessed. CONCLUSION: The improvement in AD severity with probiotic **treatment** was associated with significant increases in the capacity for Th1 IFN-gamma responses and altered responses to skin and enteric flora. This effect was still evident 2 months after the supplementation was ceased. The lack of consistent effects on **allergen**-specific responses suggests that the effects of probiotics may be mediated through other independent pathways, which need to be explored further.

L12 ANSWER 3 OF 12 MEDLINE on STN

2005202511. PubMed ID: 15836761. Nasal exposure to Staphylococcal enterotoxin enhances the development of allergic rhinitis in mice. Okano M; Hattori H; Yoshino T; Sugata Y; Yamamoto M; Fujiwara T; Satoskar A A; Satoskar A R; Nishizaki K. (Otolaryngology-Head & Neck Surgery, Okayama University Graduate School of Medicine and Dentistry, Okayama, Japan.. mokano@cc.okayama-u.ac.jp) . Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology, (2005 Apr) Vol. 35, No. 4, pp. 506-14. Journal code: 8906443. ISSN: 0954-7894. Pub. country: England: United Kingdom. Language: English.

AB BACKGROUND: Staphylococcal enterotoxins (SEs) appear to play a role in the pathogenesis of allergic disease. However, little is known whether the

nasal exposure to SE affects the development of allergic rhinitis (AR). OBJECTIVE: We sought to determine the in vivo effect of nasal exposure to SE on the development of AR using mouse model. METHODS: BALB/c mice were intranasally sensitized with Schistosoma mansoni egg antigen (SmEA) in the presence or absence of staphylococcal **enterotoxin B** (SEB). Control mice were intranasally sensitized with either SEB or SmEA alone. The production of antigen-specific antibodies including IgE, nasal eosinoplilia and cytokines by nasal mononuclear cells was compared among mice that had or had not received SEB **treatment**. RESULTS: Nasal exposure to SEB enhanced the development of AR in SmEA-sensitized mice, as manifested by SmEA-specific IgE production, nasal eosinophilia, and IL-4 and IL-5 production by nasal mononuclear cells after Ag challenge. This **treatment** also elicited IFN-gamma production by SmEA-primed cells. In addition, these mice produced SEB-specific IgE whereas mice treated with SEB without SmEA sensitization did not produce SEB-specific IgE or demonstrate nasal eosinophilia. CONCLUSION: These results suggest that the nasal exposure to SEB enhances susceptibility to AR although the exposure to SE solely does not induce AR.

L12 ANSWER 4 OF 12 EMBASE COPYRIGHT (c) 2006 Elsevier B.V. All rights reserved on STN

2005192839 EMBASE Persistent skin colonization with Staphylococcus aureus in atopic dermatitis: Relationship to clinical and immunological parameters.
Guzik T.J.; Bzowska M.; Kasprowicz A.; Czerniawska-Mysik G.; Wojcik K.; Szmyd D.; Adamek-Guzik T.; Pryjma J.. Prof. T.J. Guzik, Department of Immunology, Faculty of Biotechnology, Jagiellonian University, Gronostajowa 7, 30-387 Cracow, Poland. tguzik@well.ox.ac.uk. Clinical and Experimental Allergy Vol. 35, No. 4, pp. 448-455 2005.
Refs: 36.
ISSN: 0954-7894. CODEN: CLEAEN
Pub. Country: United Kingdom. Language: English. Summary Language: English.
Entered STN: 20050519. Last Updated on STN: 20050519

AB Background: Staphylococcus aureus has important implications for the pathogenesis of atopic dermatitis (AD). In some patients S. aureus can be eradicated from the skin during anti-inflammatory **treatment**, while in others bacterial colonization is persistent. Potential mechanisms and features of these two distinct groups of patients are not known. Objective: Accordingly, we studied relationships between the ability to eliminate S. aureus during an anti-inflammatory **treatment** and selected clinical and immunological features. Methods: Quantitative assessment of S. aureus on the skin, in nasal vestibule and throat, serum IgE levels, CD4/CD8 T-cell ratio, lymphocyte proliferation and phagocyte oxidative burst were determined during the exacerbation and after 4 and 12 weeks of the **treatment** using topical steroid and oral antihistamine in 34 patients with AD. Results: S. aureus was found on the skin of all 34 patients during exacerbation. Disease severity scoring of atopic dermatitis (SCORAD) correlated with the density of bacteria. **Treatment** with oral antihistamine and topical steroid resulted in a significant alleviation of symptoms, which correlated with the elimination of S. aureus from the skin in 70% of patients. In the remaining 30% of patients, dense (more than 10(10)/cm (2)) S. aureus skin colonization, persisted despite the **treatment** . Patients with persistent S. aureus presented with higher serum IgE levels, lower lymphocyte proliferation in response to staphylococcal **enterotoxin B**, phytohaemagluttinin and anti-CD3. Persistence of S. aureus was more common in men. Conclusions: Patients with AD differ in the ability to clear S. aureus from the skin during anti-inflammatory **treatment**, which appears to be related to the abnormalities in immunological parameters. Local antibiotic therapy should be considered only in patients with persistent S. aureus colonization. .COPYRGT. 2005 Blackwell Publishing Ltd.

L12 ANSWER 5 OF 12 CAPLUS COPYRIGHT 2006 ACS on STN
2003:633041 Document No. 139:178680 Compound and method for the prevention and/or the **treatment** of allergy. Saint-Remy, Jean-Marie; Jacquemin, Marc (Belg.). U.S. Pat. Appl. Publ. US 2003152581 A1 20030814, 26 pp., Cont.-in-part of U.S. 6,602,509. (English). CODEN: USXXCO. APPLICATION: US 2002-237656 20020910. PRIORITY: EP 1998-870167 19980730; US 1999-362731 19990729.

AB The present invention is related to a compound for the prevention and/or the **treatment** of allergy consisting of: at least one **allergen** antigenic determinant which is recognized by a B cell or an antibody secreted by a B cell of a non-atopic individual to said **allergen**, and at least one antigenic determinant of an antigen different from said **allergen** which triggers T cell activation.

L12 ANSWER 6 OF 12 MEDLINE on STN
2001489858. PubMed ID: 11531830. Impaired responses of peripheral blood mononuclear cells to T-cell stimulants in alopecia areata patients with a poor response to topical immunotherapy. Yoshino T; Asada H; Ando Y; Fujii H; Yamaguchi Y; Yoshikawa K; Itami S. (Department of Dermatology, Osaka University Graduate School of Medicine, 2-2, Yamadaoka, Suita-shi, Osaka 565-0871, Japan.) The British journal of dermatology, (2001 Sep) Vol. 145, No. 3, pp. 415-21. Journal code: 0004041. ISSN: 0007-0963. Pub. country: England: United Kingdom. Language: English.

AB BACKGROUND: Topical immunotherapy with a contact **allergen** is effective in alopecia areata (AA). However, the mechanism of the effect is still unknown, and pretreatment prediction of the outcome of therapy in each patient remains difficult. OBJECTIVES: To predict the clinical effect of this therapy in AA patients, we investigated the relationship between clinical responses to topical immunotherapy and in vitro proliferative responses of peripheral blood mononuclear cells (PBMC) to T-cell stimulants. METHODS: PBMC were taken from 67 AA patients before or during diphenylcyclopropenone immunotherapy and from 14 healthy controls, and proliferative responses to phytohaemagglutinin and staphylococcal **enterotoxin B** were evaluated by measuring [3H]-thymidine incorporation. RESULTS: PBMC from the AA patients with a good clinical response to immunotherapy showed a normal level of proliferation, whereas PBMC from the poor responders showed a markedly suppressed proliferative response and interleukin (IL)-2 production, but increased IL-4 production compared with the controls. CONCLUSIONS: The proliferative response of PBMC to T-cell stimulants may be one of the indicators of the clinical effect of topical immunotherapy for AA.

L12 ANSWER 7 OF 12 CAPLUS COPYRIGHT 2006 ACS on STN
2000:104519 Document No. 132:165114 Compound and method for the prevention and/or the **treatment** of allergy. Saint-Remy, Jean-Marie; Jacquemin, Marc (UCB S. A., Belg.). PCT Int. Appl. WO 2000006694 A2 20000210, 50 pp. DESIGNATED STATES: W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1999-BE92 19990720. PRIORITY: EP 1998-870167 19980730.

AB The present invention is related to a compound for the prevention and/or the **treatment** of allergy consisting of: at least one **allergen** antigenic determinant which is recognized by a B cell or an antibody secreted by a B cell of a non-atopic individual to said **allergen**, and at least one antigenic determinant of an antigen different from said **allergen** which triggers T cell activation. Thus, peptides or proteins containing T cell epitope of tetanus toxoid and/or B cell epitope of Der p II **allergen**, or polypeptide containing T cell epitope of

influenza A virus and B cell epitope of Der p I **allergen** were prepared for administration by gene transfer technol. through adenoviral vehicle, or by oral through food (e.g. acidified whey milk).

L12 ANSWER 8 OF 12 SCISEARCH COPYRIGHT (c) 2006 The Thomson Corporation on STN

2000:124994 The Genuine Article (R) Number: 282PJ. Role of staphylococcal superantigens in atopic dermatitis: from colonization to inflammation. Taskapan M O; Kumar P (Reprint). Louisiana State Univ, Hlth Sci Ctr, Dept Med, Sect Allergy & Clin Immunol, 1542 Tulane Ave, New Orleans, LA 70112 USA (Reprint); Louisiana State Univ, Hlth Sci Ctr, Dept Med, Sect Allergy & Clin Immunol, New Orleans, LA 70112 USA; Louisiana State Univ, Hlth Sci Ctr, Dept Allergy & Clin Immunol, New Orleans, LA USA. ANNALS OF ALLERGY ASTHMA & IMMUNOLOGY (JAN 2000) Vol. 84, No. 1, pp. 3-10. ISSN: 1081-1206. Publisher: AMER COLL ALLERGY ASTHMA IMMUNOLOGY, 85 WEST ALGONQUIN RD SUITE 550, ARLINGTON HTS, IL 60005 USA. Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Objective: This review article has been prepared in order to enable the readers to understand the role of staphylococcal superantigens (SsAgs) in atopic dermatitis (AD).

Data sources: MEDLINE literature search was performed for obtaining references. Recent reviews, research articles, poster presentations, and letters (to the editor) were meticulously reviewed.

Results: (1) SsAgs contribute to the pathogenesis of cutaneous inflammation in AD with five potential mechanisms:

Direct stimulation of antigen presenting cells (APCs) and keratinocytes

Stimulation of T cell proliferation [superantigenic binding to T eel receptor (TCR)].

Expansion of skin-homing cutaneous lymphocyte antigen (CLA) (+) T cells

The role of superantigens as **allergens**

Reduction of apoptosis

(2) Effectiveness of antibiotic therapy in AD patients without signs of bacterial infection is still under discussion. If signs of skin infection are present, antibiotic therapy (topical/oral) may help exacerbations of AD. Prolonged topical/oral antibiotic therapy, however, may cause development of antibiotic-resistant strains of Staphylococcus aureus (SA).

Conclusions: Atopic dermatitis is a genetically determined, chronically relapsing, inflammatory skin disease which has many aspects and a complex immunopathogenesis involving both immediate and cellular immune responses. While the pathogenic role of SsAgs may not be of primary importance, SsAgs appear to be one of the important triggering factors that contribute to the cutaneous inflammation in AD. We suggest that staphylococcal colonization does not always mean SsAg-mediated inflammation, and anti-staphylococcal **treatment** should be considered in cases with signs of bacterial infection.

L12 ANSWER 9 OF 12 MEDLINE on STN

1998165263. PubMed ID: 9506440. A human-SCID mouse model for allergic immune response bacterial superantigen enhances skin inflammation and suppresses IgE production. Herz U; Schnoy N; Borelli S; Weigl L; Kasbohrer U; Daser A; Wahn U; Kottgen E; Renz H. (Department of Clinical Chemistry, Virchow-Klinikum of the Humboldt-Universitat, Berlin, Germany.) The Journal of investigative dermatology, (1998 Mar) Vol. 110, No. 3, pp. 224-31. Ref: 41. Journal code: 0426720. ISSN: 0022-202X. Pub. country: United States. Language: English.

AB Chronic skin colonization with Staphylococcus aureus is a well-known feature in atopic dermatitis. The aim of this study was to develop a human-SCID mouse model to analyze the possible role of bacterial superantigens in human allergic immune responses under in vivo conditions. SCID mice were reconstituted with peripheral blood mononuclear cells

(between 2 and 9 x 10(7) cells per mouse) from atopic dermatitis patients sensitized to house dust mite **allergen** (Der p). Total and Der p specific antibody production required the following conditions: (i) injection of Der p; (ii) presence of CD14+ antigen-presenting cells; and (iii) IL-4 as shown by the inhibitory effect of human soluble IL-4 receptor on immunoglobulin E production. This model was used to study the immunomodulatory effects of the superantigen staphylococcal **enterotoxin B** in comparison with Der p. In intraperitoneally reconstituted human-SCID mice, topical **treatment** was ineffective in inducing skin inflammation. Therefore, additionally to intraperitoneal transfer, peripheral blood mononuclear cells from atopic donors were also injected intradermally. Such reconstituted SCID mice were then exposed via the skin to either Der p, staphylococcal **enterotoxin B**, or a combination of both. Maximal effects on epidermal inflammation and dermal T cell infiltration were obtained with staphylococcal **enterotoxin B** and Der p. Staphylococcal **enterotoxin B** alone was less effective and Der p only stimulated dermal T cell infiltration. These findings support the hypothesis that bacterial superantigens can act as trigger factors in allergic skin inflammation.

L12 ANSWER 10 OF 12 MEDLINE on STN

96107592. PubMed ID: 8589922. Prevention and reversal of superantigen-induced anergy by contact **allergen** exposure. Saloga J; Enk A H; Becker D; Spieles S; Bellinghausen I; Knop J. (Department of Dermatology, University of Mainz, Germany.) Experimental dermatology, (1995 Oct) Vol. 4, No. 5, pp. 308-12. Journal code: 9301549. ISSN: 0906-6705. Pub. country: Denmark. Language: English.

AB The superantigen Staphylococcal **enterotoxin B** (SEB) and the contact **allergen** 2,4-dinitrofluorbenzene (DNFB) both react with V beta 8+ T-cells delivering distinct signals. Pre-**treatment** with DNFB painted onto the same skin site where SEB was to be injected, prevented the induction of anergy in V beta + T-cells that was otherwise induced after SEB had been injected intradermally over a period of 2 weeks. Application of the irritant sodium dodecyl sulfate (SDS) instead of DNFB did not exert this effect. Application of DNFB at a site distant from the site where SEB was injected resulted in a much weaker inhibitory influence on the induction of anergy by SEB. Established anergy of V beta 8+ T-cells (proliferative non-responsiveness to SEB in vitro that could be overcome by addition of exogenous interleukin 2 (IL-2)) could be largely reversed by repeated cutaneous exposure to DNFB painted to the site where SEB had been injected before. The moderate decrease of V beta 8+ T-cells normally induced by SEB-**treatment** was also partially prevented by DNFB pre-**treatment**. The data indicate the importance of the sequence of signals delivered to T cells and the plasticity of the responsiveness of this cell type.

L12 ANSWER 11 OF 12 MEDLINE on STN

96026726. PubMed ID: 8535616. Contact sensitizers modulate mechanisms of receptor-mediated endocytosis but not fluid-phase endocytosis in murine epidermal Langerhans cells. Becker D; Lempertz U; Enk A; Saloga J; Knop J. (Hautklinik der Johannes Gutenberg-Universitat, Mainz, Germany.) Experimental dermatology, (1995 Aug) Vol. 4, No. 4 Pt 1, pp. 211-7. Journal code: 9301549. ISSN: 0906-6705. Pub. country: Denmark. Language: English.

AB In order to define the influence of contact **allergens** on the fluid-phase endocytosis (FPE) of soluble molecules of murine epidermal Langerhans cells (LC), we studied the internalization of FITC-labeled bovine serum albumin (FITC-BSA), TRITC-labeled dextrane (TRITC-DEX) as well as horseradish peroxidase by LC. A 3-parameter flow-cytometric technique was performed for quantification of internalized FITC-BSA in LC using quantum red-labeled reagents for detection of Ia-antigen expression

by LC and propidium iodide for exclusion of dead cells from analysis. A temperature-dependent rapid accumulation of FITC-BSA was noticed in time-course studies reaching a plateau between 1 and 2 h of in vitro culture at 37 degrees C. The quantity of FPE under stimulation with phorbol 12-myristate 13-acetate (PMA), concanavalin A (Con A), staphylococcal **enterotoxin B** (SEB) and contact sensitizers (DNFB, Kathon CG, K2Cr2O7) as well as the irritant SLS was determined. **Treatment** of LC with PMA and Con A resulted in a significant increase of total FITC-BSA uptake. The contact sensitizers as well as SEB and SLS failed to mediate augmented fluid-phase endocytosis. By use of the pH-insensitive soluble marker, TRITC-DEX and a microscope photometer for evaluation these findings could be confirmed. This excluded any artificial influence of differences in pH values in endocytotic compartments which might have influenced the fluorescence intensity of the pH-sensitive fluorochrome FITC. For qualitative analysis of FPE, the intracellular distribution of internalized horseradish peroxidase in LC was studied. An aggregated pattern became apparent in untreated LC and did not change under stimulation with any of the substances used. (ABSTRACT TRUNCATED AT 250 WORDS)

L12 ANSWER 12 OF 12 MEDLINE on STN

95104305. PubMed ID: 7805743. Inhibition of the development of immediate hypersensitivity by staphylococcal **enterotoxin B**.
Saloga J; Lack G; Bradley K; Renz H; Larsen G; Leung D Y; Gelfand E W. (Division of Basic Sciences, National Jewish Center for Immunology and Respiratory Medicine, Denver, CO 80206.) European journal of immunology, (1994 Dec) Vol. 24, No. 12, pp. 3140-7. Journal code: 1273201. ISSN: 0014-2980. Pub. country: GERMANY: Germany, Federal Republic of. Language: English.

AB We investigated the ability of staphylococcal **enterotoxin B** (SEB) to modify the immediate hypersensitivity response induced in BALB/c mice following sensitization to ovalbumin (OVA), a response mediated by OVA-reactive V beta 8 T cells. Mice were sensitized by skin painting with OVA every second day over a period of 2 weeks. SEB, a potent activator of V beta 8+ T cells, was administered at the same site where OVA was applied (skin of the lower abdomen) following two different protocols. In protocol (A) SEB was injected intradermally 1 day before painting with OVA and on day 7; in protocol B, SEB was injected each time OVA was applied to the skin (eight times). SEB (but not SEA) altered the development of immediate hypersensitivity to OVA, as demonstrated by the reduction in **allergen**-specific IgE, decreased OVA-specific immediate skin test responsiveness, and prevented the development of increased airways responsiveness after bronchial challenge with OVA. Injections of SEB did not alter the proliferative responses of local draining lymph node cells or spleen mononuclear cells to OVA, indicating that administration of SEB did not inhibit the sensitization of OVA, but shifted the immune response away from an immediate type response (IgE/IgG1) to IgG2a, IgG2b and IgG3. Although both protocols of SEB **treatment** did not lead to a major deletion of the V beta 8 T cell population, they did reduce the proliferative response of V beta 8+ T cells to OVA. These data indicate that the bacterial toxin SEB is capable of modifying the immediate hypersensitivity response induced by OVA by altering the functional capacity of antigen-reactive V beta 8 T cells.

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L14 ANSWER 1 OF 3 EMBASE COPYRIGHT (c) 2006 Elsevier B.V. All rights reserved on STN

2005433611 EMBASE A murine model of ulcerative colitis: Induced with sinusitis-derived superantigen and food **allergen**. Yang P.-C.; Wang C.-S.; An Z.-Y.. P.-C. Yang, Department of Pathology and Molecular Medicine, McMaster University, Hamilton, Ont., Canada. yangp@mcmaster.ca. BMC Gastroenterology Vol. 5, pp. 11p 3 Mar 2005.
Refs: 38.
ISSN: 1471-230X. CODEN: BGMABE
Pub. Country: United Kingdom. Language: English. Summary Language: English.
Entered STN: 20051013. Last Updated on STN: 20051013

AB Background: The etiology of ulcerative colitis (UC) is to be understood. The basic pathological feature of UC is intestinal chronic inflammation. Superantigen, such as Staphylococcus **enterotoxin B** (SEB), is reported to compromise intestinal barrier function by increasing epithelial permeability and initiate inflammation in the intestinal mucosa. Inasmuch as anatomic position of the sinus, chronic sinusitis-derived SEB may follow the secretion and to be swallowed down to the gastrointestinal tract and induce lesions to the intestinal mucosa. Methods: Sinus wash fluid (SWF, containing SEB) was collected from a group of patients with both chronic sinusitis (CS) and UC. A group of mice were sensitized to ovalbumin (OVA) in the presence of SWF. The sensitized mice were challenged with the specific antigen OVA. The inflammatory status of the colonic tissue was determined with histology, serology and electron microscopy. Using horseradish peroxidase (HRP) as a tracer, another group of mice was stimulated with SWF for 2 hours. The HRP activity was detected in the colonic tissue with enzymatic approaches and electron microscopy. Results: Epithelial hyperpermeability in colonic epithelium was induced by stimulating with SWF. The HRP activity in the colonic mucosa was almost 11 times more in the SWF treated group (3.2 ± 0.6 μg/g tissue) than the control group (0.3 ± 0.1 μg/g tissue). Mice were sensitized using a mixture of SWF and OVA (serum OVA-specific IgE was detected with a highest titer as 1:64). Challenge with OVA induced extensive inflammation in the colonic mucosa by showing (1) marked degranulation in mast cells (MC, 46.3 ± 4.5%) and eosinophils (Eo, 55.7 ± 4.2%); (2) inflammatory cell infiltration (MC = 145.2 ± 11.4; Eo = 215.8 ± 12.5; mononuclear cell = 258.4 ± 15.3/mm(2) tissue); (3) increased MPO activity (12.9 ± 3.2 U/g tissue) and inflammatory scores (1.8 ± 0.3); (4) **mucosal** surface ulcers; (5) edema in the lamina propria; (6) bacterial translocation and abscess formation in the subepithelial region. Conclusion: Introducing Sinusitis-derived SEB-containing SWF to the gastrointestinal tract compromised colonic **mucosal** barrier function increasing epithelial permeability to luminal macromolecular protein in mice. The SWF facilitated colonic **mucosal** sensitization to luminal antigen. Multiple challenging the sensitized colonic mucosa with specific antigen OVA induced inflammation, induced a condition similar to human ulcerative colitis. .COPYRGT. 2005 Yang et al; licensee BioMed Central Ltd.

L14 ANSWER 2 OF 3 MEDLINE on STN

2005164997. PubMed ID: 15745456. A murine model of ulcerative colitis: induced with sinusitis-derived superantigen and food **allergen**. Yang Ping-Chang; Wang Chang-Sheng; An Zi-Yuan. (Department of Pathology and Molecular Medicine, McMaster University, Hamilton, Ontario, Canada.. yangp@mcmaster.ca) . BMC gastroenterology [electronic resource], (2005) Vol. 5, No. 1, pp. 6. Electronic Publication: 2005-03-03. Journal code: 100968547. E-ISSN: 1471-230X. Pub. country: England: United Kingdom. Language: English.

AB BACKGROUND: The etiology of ulcerative colitis (UC) is to be understood. The basic pathological feature of UC is intestinal chronic inflammation. Superantigen, such as Staphylococcus **enterotoxin B**

(SEB), is reported to compromise intestinal barrier function by increasing epithelial permeability and initiate inflammation in the intestinal mucosa. Inasmuch as anatomic position of the sinus, chronic sinusitis-derived SEB may follow the secretion and to be swallowed down to the gastrointestinal tract and induce lesions to the intestinal mucosa. METHODS: Sinus wash fluid (SWF, containing SEB) was collected from a group of patients with both chronic sinusitis (CS) and UC. A group of mice were sensitized to ovalbumin (OVA) in the presence of SWF. The sensitized mice were challenged with the specific antigen OVA. The inflammatory status of the colonic tissue was determined with histology, serology and electron microscopy. Using horseradish peroxidase (HRP) as a tracer, another group of mice was stimulated with SWF for 2 hours. The HRP activity was detected in the colonic tissue with enzymatic approaches and electron microscopy. RESULTS: Epithelial hyperpermeability in colonic epithelium was induced by stimulating with SWF. The HRP activity in the colonic mucosa was almost 11 times more in the SWF treated group (3.2 +/- 0.6 microg/g tissue) than the control group (0.3 +/- 0.1 microg/g tissue). Mice were sensitized using a mixture of SWF and OVA (serum OVA-specific IgE was detected with a highest titer as 1:64). Challenge with OVA induced extensive inflammation in the colonic mucosa by showing (1) marked degranulation in mast cells (MC, 46.3 +/- 4.5%) and eosinophils (Eo, 55.7 +/- 4.2%); (2) inflammatory cell infiltration (MC = 145.2 +/- 11.4; Eo = 215.8 +/- 12.5; mononuclear cell = 258.4 +/- 15.3/mm2 tissue); (3) increased MPO activity (12.9 +/- 3.2 U/g tissue) and inflammatory scores (1.8 +/- 0.3); (4) **mucosal** surface ulcers; (5) edema in the lamina propria; (6) bacterial translocation and abscess formation in the subepithelial region. CONCLUSION: Introducing Sinusitis-derived SEB-containing SWF to the gastrointestinal tract compromised colonic **mucosal** barrier function increasing epithelial permeability to luminal macromolecular protein in mice. The SWF facilitated colonic **mucosal** sensitization to luminal antigen. Multiple challenging the sensitized colonic mucosa with specific antigen OVA induced inflammation, induced a condition similar to human ulcerative colitis.

L14 ANSWER 3 OF 3 CAPLUS COPYRIGHT 2006 ACS on STN

2005:298339 Document No. 143:285031 A murine model of ulcerative colitis: Induced with sinusitis-derived superantigen and food **allergen**. Yang, Ping-Chang; Wang, Chang-Sheng; An, Zi-Yuan (Department of Pathology and Molecular Medicine, McMaster University, Hamilton, ON, Can.). BMC Gastroenterology, 5, No pp. given (English) 2005. CODEN: BGMABE. ISSN: 1471-230X. URL: http://www.biomedcentral.com/content/pdf/1471-230X-5-6.pdf Publisher: BioMed Central Ltd..

AB The etiol. of ulcerative colitis (UC) is to be understood. The basic pathol. feature of UC is intestinal chronic inflammation. Superantigen, such as Staphylococcus **enterotoxin B** (SEB), is reported to compromise intestinal barrier function by increasing epithelial permeability and initiate inflammation in the intestinal mucosa. Inasmuch as anat. position of the sinus, chronic sinusitis-derived SEB may follow the secretion and to be swallowed down to the gastrointestinal tract and induce lesions to the intestinal mucosa. Sinus wash fluid (SWF, containing SEB) was collected from a group of patients with both chronic sinusitis (CS) and UC. A group of mice were sensitized to ovalbumin (OVA) in the presence of SWF. The sensitized mice were challenged with the specific antigen OVA. The inflammatory status of the colonic tissue was determined with histol., serol. and electron microscopy. Using horseradish peroxidase (HRP) as a tracer, another group of mice was stimulated with SWF for 2 h. The HRP activity was detected in the colonic tissue with enzymic approaches and electron microscopy. Epithelial hyperpermeability in colonic epithelium was induced by stimulating with SWF. The HRP activity in the colonic mucosa was almost 11 times more in the SWF treated group (3.2±0.6 μg/g tissue) than the control group (0.3±0.1 μg/g tissue). Mice were sensitized using a mixture of SWF and OVA (serum OVA-specific IgE was detected with a highest titer as

1:64). Challenge with OVA induced extensive inflammation in the colonic mucosa by showing (1) marked degranulation in mast cells (MC, 46.3±4.5%) and eosinophils (Eo, 55.7±4.2%); (2) inflammatory cell infiltration (MC = 145.2±11.4; Eo = 215.8±12.5; mononuclear cell = 258.4±15.3/mm2 tissue); (3) increased MPO activity (12.9±3.2 U/g tissue) and inflammatory scores (1.8±0.3); (4) **mucosal** surface ulcers; (5) edema in the lamina propria; (6) bacterial translocation and abscess formation in the subepithelial region. Introducing Sinusitis-derived SEB-containing SWF to the gastrointestinal tract compromised colonic **mucosal** barrier function increasing epithelial permeability to luminal macromol. protein in mice. The SWF facilitated colonic **mucosal** sensitization to luminal antigen. Challenging the sensitized colonic mucosa with specific antigen OVA induced inflammation, induced a condition similar to human ulcerative colitis.

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L18 ANSWER 1 OF 1 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN
DUPLICATE 1
1994:246123 Document No.: PREV199497259123. **Cholera toxin** (**CTX**) promotes IgE antibody and **allergy** during oral immunization. Snider, D. P.; Marshall, J. S.; Perdue, M. H.; Liang, H.. Dep. Pathology, MVIP, McMaster Univ., Hamilton, ON L8N 3Z5, Canada. FASEB Journal, (1994) Vol. 8, No. 4-5, pp. A282.
Meeting Info.: Experimental Biology 94, Parts I and II. Anaheim, California, USA. April 24-28, 1994.
CODEN: FAJOEC. ISSN: 0892-6638. Language: English.

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L22 ANSWER 1 OF 27 MEDLINE on STN DUPLICATE 1
2006197057. PubMed ID: 16461395. Gas phase characterization of the noncovalent quaternary structure of **cholera toxin** and the **cholera toxin** B subunit pentamer. Williams

Jonathan P; Smith Daniel C; Green Brian N; Marsden Brian D; Jennings Keith R; Roberts Lynne M; Scrivens James H. (Department of Biological Sciences, University of Warwick, Coventry CV4 7AL, United Kingdom.) Biophysical journal, (2006 May) Vol. 90, No. 9, pp. 3246-54. Electronic Publication: 2006-02-03. Journal code: 0370626. ISSN: 0006-3495. Pub. country: United States. Language: English.

AB **Cholera toxin** (**CTx**) is an AB(5) cytotonic protein that has medical relevance in cholera and as a novel **mucosal** adjuvant. Here, we report an analysis of the noncovalent homopentameric complex of **CTx** B chain (**CTx** B(5)) using electrospray ionization triple quadrupole mass spectrometry and tandem mass spectrometry and the analysis of the noncovalent hexameric holotoxin usingelectrospray ionization time-of-flight mass spectrometry over a range of pH values that correlate with those encountered by this toxin after cellular uptake. We show that noncovalent interactions within the toxin assemblies were maintained under both acidic and neutral conditions in the gas phase. However, unlike the related Escherichia coli Shiga-like toxin B(5) pentamer (SLTx B), the **CTx** B(5) pentamer was stable at low pH, indicating that additional interactions must be present within the latter. Structural comparison of the **CTx** B monomer interface reveals an additional alpha-helix that is absent in the SLTx B monomer. In silico energy calculations support interactions between this helix and the adjacent monomer. These data provide insight into the apparent stabilization of **CTx** B relative to SLTx B.

L22 ANSWER 2 OF 27 MEDLINE on STN DUPLICATE 2

2006232624. PubMed ID: 16275129. Synthesis and assembly of an adjuvanted Porphyromonas gingivalis fimbrial antigen fusion protein in plants. Shin Eun-Ah; Lee Jin-Yong; Kim Tae-Geum; Park Yong Keun; Langridge William H R. (Department of Biochemistry and Microbiology, Center for Molecular Biology and Gene Therapy, School of Medicine, Loma Linda University, Loma Linda, CA 92350, USA; School of Life Science and Biotechnology, Korea University, Seoul 130-700, Republic of Korea.) Protein expression and purification, (2006 May) Vol. 47, No. 1, pp. 99-109. Electronic Publication: 2005-10-05. Journal code: 9101496. ISSN: 1046-5928. Pub. country: United States. Language: English.

AB The gram-negative anaerobic oral bacterium Porphyromonas gingivalis initiates periodontal disease by binding to saliva-coated oral surfaces. To assess whether edible plants can synthesize biologically active P. gingivalis fimbrial antigen, for application as an oral vaccine, a cDNA fragment encoding the C-terminal binding portion of P. gingivalis fimbrial protein (FimA), was cloned into a plant expression vector immediately downstream of a cDNA fragment encoding the **cholera toxin** B subunit (CTB). The chimeric plasmid was transferred into potato (Solanum tuberosum) cells and the ctb-fimA cDNA fragment detected in transformed leaf genomic DNA by PCR amplification methods. A novel protein band of 21kDa was detected in transformed potato tuber extracts by immunoblot analysis. Oligomeric CTB-FimA (266-337) fusion protein was identified in the extracts through the binding of anti-**CTX** and anti-native fimbriae antibodies. The pentameric structure of CTB-FimA fusion protein was confirmed by ELISA measurements of G(M1) ganglioside receptor binding. Quantification of the CTB-FimA fusion protein by ELISA indicated that the chimeric protein made up about 0.33% of total soluble tuber protein. The biosynthesis of immunologically detectable CTB-FimA fusion proteins and the assembly of fusion protein monomers into biologically active pentamers in transformed potato tuber tissues demonstrate the feasibility of synthesizing adjuvanted fimbrial protein in edible plants for development of adjuvanted **mucosal** vaccines against P. gingivalis generated periodontal disease.

L22 ANSWER 3 OF 27 CAPLUS COPYRIGHT 2006 ACS on STN

2005:1132637 Document No. 143:417259 Recombinant uropathogenic E. coli antigen fused to CTXA2B or LTXA2B proteins for use as vaccine against

urinary tract infections. Pyo, Suhk-Neung; Lee, Yong-Hwa (Sungkyunkwan University, S. Korea). U.S. Pat. Appl. Publ. US 2005232938 A1 20051020, 35 pp. (English). CODEN: USXXCO. APPLICATION: US 2004-877670 20040625. PRIORITY: KR 2004-26900 20040419.

AB Disclosed is a novel vaccine against Escherichia coli responsible for urinary tract infections. The vaccine is a recombinant chimeric protein which is prepared by linking by genetic recombination a gene encoding an antigenic determinant of uropathogenic E. coli (such as FimH) to a CTXA2B gene encoding nontoxic A2 and B subunits of Vibrio cholerae **cholera toxin** (**CTX**) or an LTXA2B gene encoding nontoxic A2 and B subunits of E. coli heat-labile enterotoxin, wherein a translation product of the CTXA2B or LTXA2B gene serves as an immunogenic adjuvant stimulating **mucosal** immune responses, expressing the resulting recombinant gene in E. coli, and isolating and purifying an expressed recombinant fusion protein. The recombinant chimeric protein is useful as an oral vaccine with mild side effects and excellent vaccination efficiency against uropathogenic E. coli. Thus, the chimeric vaccine protein can remarkably reduce recurrence of urinary tract infections, prevent occurrence of antibiotic-resistant bacteria, and replace the conventional chemotherapy for urinary tract infections. Also, the chimeric vaccine protein has other advantages of being capable of being produced and commercialized in a short period with relatively low costs, and being easily modified by replacing its genetic constituents with other genes to provide various vaccines.

L22 ANSWER 4 OF 27 MEDLINE on STN DUPLICATE 3

2005179595. PubMed ID: 15812247. **Cholera toxin** potentiates influences of IFN-gamma through activation of NF-kappaB and release of tumor necrosis factor-alpha. Blumberg Richard S; Pitman Richard S; Taylor Cormac T; Colgan Sean P. (Division of Gastroenterology, Brigham and Women's Hospital and Harvard Medical School, 20 Shattuck Street, Boston, MA 02115, USA.) Journal of interferon & cytokine research : the official journal of the International Society for Interferon and Cytokine Research, (2005 Apr) Vol. 25, No. 4, pp. 209-19. Journal code: 9507088. ISSN: 1079-9907. Pub. country: United States. Language: English.

AB **Cholera toxin** (**Ctx**) is a potent adjuvant in the **mucosal** immune system. Previous studies have indicated that **Ctx** induces intestinal interferon-gamma (IFN-gamma) production and that adjuvant properties require activation of the IFN-gamma receptor (IFNGR). Thus, we hypothesized that **Ctx** potentiates IFN-gamma responses in intestinal epithelia. Initial studies suggested that **Ctx** enhances IFN-gamma-mediated barrier disruption in cultured intestinal epithelia. This response was attributable to liberation of a soluble mediator into conditioned supernatants, subsequently identified as tumor necrosis factor-alpha (TNF-alpha). Extensions of these findings revealed that the **Ctx** A subunit induces transcriptional activation of proinflammatory genes in addition to TNF-alpha (interleukin-8 [IL- 8], intracellular adhesion molecule-1 [ICAM-1], and IL-6) and that such transactivation is mediated by the transcriptional regulator NF-kappaB. We conclude that **Ctx** elicits a proinflammatory phenotype in intestinal epithelia and that potentiation of IFN-gamma-mediated barrier disruption by TNF-alpha may contribute to the overall adjuvant properties of **Ctx**.

L22 ANSWER 5 OF 27 EMBASE COPYRIGHT (c) 2006 Elsevier B.V. All rights reserved on STN

2004025445 EMBASE Role of the W07-toxin on Vibrio cholerae-induced diarrhoea. Bhattacharyya S.; Ghosh S.; Shant J.; Ganguly N.K.; Majumdar S.. S. Ghosh, Dept. of Exp. Med. and Biotechnology, Post Grad. Inst. Med. Educ. and Res., Chandigarh 160012, India. Biochimica et Biophysica Acta - General Subjects Vol. 1670, No. 1, pp. 69-80 5 Jan 2004.
Refs: 52.
ISSN: 0304-4165. CODEN: BBGSB3

Pub. Country: Netherlands. Language: English. Summary Language: English. Entered STN: 20040220. Last Updated on STN: 20040220

AB Vibrio cholerae W07 strain isolated from a cholera epidemic in South India, lacked the **ctx** gene but could still secrete a novel toxin, the W07-toxin that could cause fluid accumulation in ligated rabbit ileal loop. The important intracellular messengers implicated in this study were Ca(2+), cyclic AMP, inositol triphosphate and protein kinase C (PKC). A number of inhibitors/channel blockers have further shown the major role of [Ca (2+)](i) in modulation of the toxin-induced cellular response. An increase in the level of reactive oxygen species (ROS) in the W07-toxin-stimulated enterocytes correlated with the decrease in the levels of antioxidant enzymes, catalase and superoxide dismutase (SOD). The reactive nitrogen intermediates (RNI) detected by measuring the levels of nitrite and citrulline, were found to be high in the enterocytes triggered with the W07-toxin, thereby indicating their role in toxin-mediated change in **mucosal** permeability. The precise role of the toxin has also been authenticated by conducting the experiments with W07-toxin preincubated in the presence of IgG(WT) (IgG isolated from antitoxin sera) or GM(1). Thus, a significant increase in the levels of second messengers and a decrease in antioxidant defenses appear to be important in mediating the fluid secretion caused by this novel toxin from V. cholerae W07. .COPYRGT. 2003 Published by Elsevier B.V.

L22 ANSWER 6 OF 27 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN

2004:292303 Document No.: PREV200400291785. Peroral Immunization of uropathogenic Escherichia coli Adhesin protein Linked to **Cholera Toxin** A2B subunits. Lee, Yonghwa [Reprint Author]; Rhee, DongKwon; Pyo, Suhkneung. College of Pharmacy, Sungkyunkwan University, 300 Chunchun-dong Jangan-gu, Suwon, Kyunggi-do, 440-746, South Korea. snpyo@skku.ac.kr. FASEB Journal, (2004) Vol. 18, No. 4-5, pp. Abst. 558.21. http://www.fasebj.org/. e-file.
Meeting Info.: FASEB Meeting on Experimental Biology: Translating the Genome. Washington, District of Columbia, USA. April 17-21, 2004. FASEB. ISSN: 0892-6638 (ISSN print). Language: English.

AB The FimH subunit of type 1-fimbriated uropathogenic Escherichia coli (UPEC) has been determined as a major cause for urinary tract infections. The chimaeric construct adhesin-LTXA2B derived from UPEC FimH adhesin genetically coupled to **cholera Toxin** (**CTX**) subunits A2 and B (CTXA2B) was expressed in E. coli as an soluble recombinant chimaeric protein. We have evaluated the efficacy of a possible vaccine antigen, FimH adhesin-CTXA2B chimaeric protein, against urinary tract infections. The protein was purified by osmotic shock and affinity chromatography. The composition of purified FimH adhesin-CTXA2B was verified by SDS/PAGE and Western blotting with antibodies to antigenic components of FimH adhesin and CTXB, and confirmed as a chimaeric protein with GM1 ganglioside binding activity and FimH adhesin epitopes by a GM1-ELISA developed using antibodies to FimH adhesin. Oral immunization of mice with FimH adhesin-CTXA2B induced higher level of **mucosal** IgA and serum IgG antibodies to FimH adhesin and to LTXB than in mice immunized with FimH adhesin or CTXA2B alone. FimH adhesin-CTXA2B was also demonstrated to be potential protective and therapeutic antigens in a mouse model infected with uropathogenic E. coli J96. Taken together, the results indicated that the genetically linked CTXA2B acts as a useful **mucosal** adjuvant, and that the FimH adhesin-CTXA2B chimaeric protein could be a potential component in future UPEC vaccine development.

L22 ANSWER 7 OF 27 MEDLINE on STN DUPLICATE 4

2003139735. PubMed ID: 12654855. **Mucosal** immunization with a genetically engineered pertussis toxin S1 fragment-**cholera toxin** subunit B chimeric protein. Lee Song F; Halperin Scott A; Salloum Danny F; MacMillan Ann; Morris Annette. (Department of Applied Oral Sciences, Faculty of Dentistry, Dalhousie University, Halifax, Nova Scotia, Canada B3H 3J5.. song.lee@dal.ca) . Infection and immunity, (2003

Apr) Vol. 71, No. 4, pp. 2272-5. Journal code: 0246127. ISSN: 0019-9567. Pub. country: United States. Language: English.

AB A chimeric protein consisting of a divalent pertussis toxin (PT) S1 fragment linked to the **cholera toxin** (**Ctx**) A(2)B fragment was constructed. The chimera induced a **mucosal** immunoglobulin A (IgA) and a serum IgG immune response to PT and CtxB in BALB/c mice following intranasal immunization. The immune sera neutralized PT in vitro. In the mouse model of Bordetella pertussis respiratory infection, the chimera-immunized animals showed a significant reduction in bacterial lung counts (P = 0.01) from that of the sham control group. Thus, a divalent S1 fragment CtxA2B chimera is an immunogenic antigen and can elicit a protective immunity.

L22 ANSWER 8 OF 27 SCISEARCH COPYRIGHT (c) 2006 The Thomson Corporation on STN DUPLICATE 5

2003:419429 The Genuine Article (R) Number: 676QZ. GM1-binding ability and immunogenicity of CTB /CS3 fusion protein expressed in E-coli. Mi K X (Reprint); Li J; Zhang Z S; Fang R X. Chinese Acad Sci, Inst Microbiol, Lab Plant Biotechnol, Beijing 100080, Peoples R China; Beijing Inst Biotechnol, Beijing, Peoples R China. PROGRESS IN BIOCHEMISTRY AND BIOPHYSICS (APR 2003) Vol. 30, No. 2, pp. 278-284. ISSN: 1000-3282. Publisher: SCIENCE CHINA PRESS, 16 DONGHUANGCHENGGEN NORTH ST, BEIJING 100717, PEOPLES R CHINA. Language: Chinese.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Enterotoxigenic Escherichia coli (ETEC) is a major pathogen that evokes acute diarrhea among children worldwide and travelers to developing countries. However, there is no ideal vaccine against it yet. In an effort to develop a subunit vaccine for ETEC, a translational fusion with **cholera toxin** B subunit (CTB) upstream of CS3 was constructed. The fusion protein synthesized in E. coli had a molecular mass of 29 ku, as expected and retained the antigenicity of both CTB and CS3 as confirmed by Western blot analysis with the polyclonal anti-**CTX** rabbit serum and the monoclonal anti-CS3 mouse serum, respectively. The 6 x His-tagged CTB/CS3 protein was purified by Ni-NTA affinity chromatography followed by renaturation. A fraction of the fusion protein could form pentamers and these pentamers retained the ability to bind GM1-ganglioside. Mice immunized by intraperitoneal injection with the fusion protein produced anti-CTB and anti-CS3 serum IgG and secretory IgA. Furthermore, it was shown that fusion to CTB increased the systemic and **mucosal** immune responses against CS3 to some extent.

L22 ANSWER 9 OF 27 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN

2003:307898 Document No.: PREV200300307898. **Mucosal cholera toxin** responses are partially and differentially mediated by 5-HT4 and Y4 receptors in the mouse descending colon. Tough, I. R. [Reprint Author]; Cox, H. M. [Reprint Author]. Centre for Neuroscience Research, King's College London, Guy's Campus, London, SE1 9RT, UK. British Journal of Pharmacology, (April 2003) Vol. 138, No. Proceedings Supplement, pp. 91P. print.

Meeting Info.: Proceedings of the British Pharmacological Society Meeting. Brighton, UK. January 08-10, 2003. British Pharmacological Society. ISSN: 0007-1188 (ISSN print). Language: English.

L22 ANSWER 10 OF 27 MEDLINE on STN DUPLICATE 6

2001248162. PubMed ID: 11292779. Escherichia coli heat-labile enterotoxin B subunit is a more potent **mucosal** adjuvant than its vlosely related homologue, the B subunit of **cholera toxin**.

Millar D G; Hirst T R; Snider D P. (Department of Pathology and Molecular Medicine, McMaster University, Hamilton, Ontario, Canada L8N 3Z5.. dmillar@uhnres.utoronto.ca) . Infection and immunity, (2001 May) Vol. 69, No. 5, pp. 3476-82. Journal code: 0246127. ISSN: 0019-9567. Pub. country: United States. Language: English.

AB Although **cholera toxin** (**Ctx**) and Escherichia coli heat-labile enterotoxin (Etx) are known to be potent **mucosal** adjuvants, it remains controversial whether the adjuvanticity of the holotoxins extends to their nontoxic, receptor-binding B subunits. Here, we have systematically evaluated the comparative adjuvant properties of highly purified recombinant EtxB and CtxB. EtxB was found to be a more potent adjuvant than CtxB, stimulating responses to hen egg lysozyme when the two were coadministered to mice intranasally, as assessed by enhanced serum and secretory antibody titers as well as by stimulation of lymphocyte proliferation in spleen and draining lymph nodes. These results indicate that, although structurally very similar, EtxB and CtxB have strikingly different immunostimulatory properties and should not be considered equivalent as prospective vaccine adjuvants.

L22 ANSWER 11 OF 27 MEDLINE on STN DUPLICATE 7

2001192726. PubMed ID: 11160664. Protective **mucosal** immunity to ocular herpes simplex virus type 1 infection in mice by using Escherichia coli heat-labile enterotoxin B subunit as an adjuvant. Richards C M; Aman A T; Hirst T R; Hill T J; Williams N A. (Department of Pathology and Microbiology, School of Medical Sciences, University of Bristol, Bristol BS8 1TD, United Kingdom.. Claire.M.Richards@bristol.ac.uk) . Journal of virology, (2001 Feb) Vol. 75, No. 4, pp. 1664-71. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB The potential of nontoxic recombinant B subunits of **cholera toxin** (rCtxB) and its close relative Escherichia coli heat-labile enterotoxin (rEtxB) to act as **mucosal** adjuvants for intranasal immunization with herpes simplex virus type 1 (HSV-1) glycoproteins was assessed. Doses of 10 microg of rEtxB or above with 10 microg of HSV-1 glycoproteins elicited high serum and **mucosal** anti-HSV-1 titers comparable with that obtained using CtxB (10 microg) with a trace (0.5 microg) of whole toxin (**Ctx**-CtxB). By contrast, doses of rCtxB up to 100 microg elicited only meager anti-HSV-1 responses. As for **Ctx**-CtxB, rEtxB resulted in a Th2-biased immune response with high immunoglobulin G1 (IgG1)/IgG2a antibody ratios and production of interleukin 4 (IL-4) and IL-10 as well as gamma interferon by proliferating T cells. The protective efficacy of the immune response induced using rEtxB as an adjuvant was assessed following ocular challenge of immunized and mock-immunized mice. Epithelial disease was observed in both groups, but the immunized mice recovered by day 6 whereas mock-immunized mice developed more severe corneal disease leading to stromal keratitis. In addition, a significant reduction in the incidence of lid disease and zosteriform spread was observed in immunized animals and there was no encephalitis compared with 95% encephalitis in mock-immunized mice. The potential of such **mucosal** adjuvants for use in human vaccines against pathogens such as HSV-1 is discussed.

L22 ANSWER 12 OF 27 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN

2002:223178 Document No.: PREV200200223178. Construction and **mucosal** immunogenicity of dimeric pertussis toxin-S1/S1 antigens genetically linked to **cholera toxin** A2/B. Salloum, D. F. [Reprint author]; Lee, S. F. [Reprint author]; Halperin, S. A. [Reprint author]. Dalhousie University, Halifax, NS, Canada. Abstracts of the General Meeting of the American Society for Microbiology, (2001) Vol. 101, pp. 335. print.
Meeting Info.: 101st General Meeting of the American Society for Microbiology. Orlando, FL, USA. May 20-24, 2001. American Society of Microbiology.
ISSN: 1060-2011. Language: English.

AB Induction of a protective humoral immune response at **mucosal** surfaces, the initial barrier to most pathogens, is not readily achieved by systemic or **mucosal** administration of vaccine antigens. Increase in the incidence of whooping cough, a vaccine preventable disease

caused by Bordetella pertussis has necessitated studies into the development of a new vaccine. In this work, the capacity of **cholera toxin** A2/B (CtxA2B) as a carrier for **mucosal** delivery of vaccine antigens was exploited to construct a chimeric fusion consisting of two in tandem copies of DNA encoding a 179 amino acid fragment of the N-terminus pertussis toxin S1 subunit. DNA encoding a non-toxic GM1-binding entity of **cholera toxin** CtxA/2B was cloned downstream of the S1/S1 fusion creating a S1/S1/CtxA2B genetic fusion. The S1/S1/CtxA2B fragment was subsequently cloned downstream to the maltose-binding protein (MBP) gene in pMALp. In-frame fusion was demonstrated by Western blotting and GM1-binding ELISA. Expression of MBP/S1/S1/CtxA2B was induced by IPTG and the chimeric protein was solubilized and isolated using 6M urea. SDS-PAGE and Western blotting confirmed isolation of the chimeric protein. GM1-binding ELISA demonstrated that the fusion protein is associating with the **Ctx** B-pentamer, forming the desired macromolecule. Intranasal administration of the MBP/S1/S1/CtxA2B chimera induced a **mucosal** (salivary sIgA) and a systemic (serum IgG) immune response to PT and CT in female BALB/c mice. In conclusion, a divalent pertussis toxin S1 fragment was successfully fused to **cholera toxin** A/2B and the chimeric protein, purified from Escherichia coli induces a **mucosal** and systemic immune response.

L22 ANSWER 13 OF 27 MEDLINE on STN DUPLICATE 8

2001206192. PubMed ID: 11222115. Peroral immunization with Helicobacter pylori adhesin protein genetically linked to **cholera toxin** A2B subunits. Kim B O; Shin S S; Yoo Y H; Pyo S. (School of Pharmacy, Sung Kyun Kwan University, Suwon, 440-746, Kyunggi-Do, South Korea.) Clinical science (London, England : 1979), (2001 Mar) Vol. 100, No. 3, pp. 291-8. Journal code: 7905731. ISSN: 0143-5221. Pub. country: England: United Kingdom. Language: English.

AB Helicobacter pylori is a major cause of gastric-associated diseases. To evaluate the efficacy of a possible vaccine antigen against H. pylori infection, the chimaeric construct adhesin--CTXA2B, derived from H. pylori adhesin genetically coupled to **cholera toxin** (**CTX**) subunits A2 and B (CTXA2B), was expressed in Escherichia coli as an insoluble recombinant chimaeric protein. The protein was then purified by denaturation, renaturation and size-exclusion chromatography. The composition of purified adhesin--CTXA2B was verified by SDS/PAGE and Western blotting with antibodies to antigenic components of adhesin and CTXB, and confirmed as a chimaeric protein with G(M1)-ganglioside binding activity and adhesin epitopes by a G(M1)-ELISA developed using antibodies to adhesin. Oral immunization of mice with adhesin--CTXA2B induced higher levels of **mucosal** IgA and serum IgG antibodies to H. pylori adhesin and to CTXB than in mice immunized with adhesin or CTXA2B alone. Adhesin--CTXA2B was also demonstrated to be a potential protective antigen in a mouse model of H. pylori infection. The immunization of mice with adhesin--CTXA2B protected 62.5% of mice infected with H. pylori SS1 strain, whereas adhesin immunization was not able to confer protection to mice. This protection may be correlated with high levels of **mucosal** IgA and serum IgG antibodies against H. pylori adhesin. Taken together, the results indicate that the genetically linked CTXA2B acts as a useful **mucosal** adjuvant, and that the adhesin-CTXA2B chimaeric protein could be a potential component in future H. pylori vaccine development.

L22 ANSWER 14 OF 27 MEDLINE on STN DUPLICATE 9

2001221761. PubMed ID: 11111925. Immune modulation by the cholera-like enterotoxin B-subunits: from adjuvant to immunotherapeutic. Williams N A. (University of Bristol, Department of Pathology and Microbiology, School of Medical Sciences, UK.. Neil.a.williams@bris.ac.uk) . International journal of medical microbiology : IJMM, (2000 Oct) Vol. 290, No. 4-5, pp. 447-53. Ref: 43. Journal code: 100898849. ISSN: 1438-4221. Pub. country:

GERMANY: Germany, Federal Republic of. Language: English.

AB **Cholera toxin** (**Ctx**) and its close relative, Escherichia coli heat-labile enterotoxin (Etx) have long been established as potent **mucosal** and systemic adjuvants. Problems arising from their inherent toxicity have, however, precluded human use. Here we describe findings which demonstrate that contrary to the established dogma the non-toxic B-subunit of Etx (EtxB) is a highly potent **mucosal** adjuvant capable of potentiating protective immunity to viral infection. The mechanisms which underlie this activity arise from an ability to trigger specific signaling processes in lymphocyte populations which modulate differentially their activation, differentiation and survival. The elucidation of these properties has led to the further use of EtxB as an agent capable of preventing the establishment of autoimmune diseases. The basis for these activities and their potential applicability to human therapies are discussed.

L22 ANSWER 15 OF 27 MEDLINE on STN DUPLICATE 10

2000445484. PubMed ID: 10994530. **Cholera toxin** and related enterotoxins: a cell biological and immunological perspective. de Haan L; Hirst T R. (Department of Pathology and Microbiology, School of Medical Sciences, University of Bristol, United Kingdom.) Journal of natural toxins, (2000 Aug) Vol. 9, No. 3, pp. 281-97. Ref: 126. Journal code: 9208016. ISSN: 1058-8108. Pub. country: United States. Language: English.

AB **Cholera toxin** (**Ctx**) from Vibrio cholerae and the closely related Escherichia coli heat-labile enterotoxin (Etx) are the primary virulence factors responsible for causing cholera and traveller's diarrhea, respectively. Studies on the mode of action of these toxins on gut epithelial cells have revealed important insights into the mechanisms of toxin uptake and trafficking in eukaryotic cells. However, of perhaps even greater fascination have been the discoveries that **Ctx** and Etx exhibit remarkable immunological properties. When either of these toxins is administered via **mucosal** routes, it triggers a potent **mucosal** and systemic anti-toxin immune response. By contrast, local or systemic immunization with other soluble protein antigens usually stimulates only a meagre immune response, or results in a state of immunological tolerance. Even more striking are the findings that when **Ctx** or Etx are mixed with heterologous antigens, they function as adjuvants, leading to stimulation of **mucosal** responses to the admixed antigen, and the abrogation of oral tolerance. In addition, recent observations have shown that the receptor-binding component of these toxins can down-regulate inflammatory diseases associated with the induction of autoimmune disorders such as rheumatoid arthritis, diabetes, and multiple sclerosis. While the underlying mechanisms responsible for these remarkable properties have yet to be resolved, it is clear that the toxins' ability to bind to cell surface receptors plays an important role in their potent immunogenicity, adjuvanticity, and immunotherapeutic properties. This review provides an overview of the latest developments within the **Ctx**/Etx field, with a special emphasis on the cell entry mechanisms and immunomodulatory action of **Ctx**/Etx and their component subunits.

L22 ANSWER 16 OF 27 CAPLUS COPYRIGHT 2006 ACS on STN

2000:883742 Document No. 135:44842 Immune modulation by the cholera-like enterotoxin B-subunits: From adjuvant to immunotherapeutic. Pitman, Richard S.; Hirst, Timothy R.; Williams, Neil A. (Division of Gastroenterology, Department of Medicine, Brigham and Women's Hospital, Boston, MA, 02115, USA). Recent Research Developments in Immunology, 1(Pt. 2), 497-511 (English) 1999. CODEN: RRDIB8. Publisher: Research Signpost.

AB A review with 59 refs. **Cholera toxin** (**Ctx**) and its close relative, Escherichia coli heat-labile enterotoxin (Etx) have long been established as potent **mucosal** and systemic

adjuvants. Problems arising from their inherent toxicity have, however, precluded human use. Here the authors describe findings which demonstrate that the non-toxic B-subunit of Etx (EtxB) is a highly potent **mucosal** adjuvant capable of potentiating protective immunity to viral infection. The mechanisms which underlie this activity arise from an ability to trigger specific signaling processes in lymphocyte populations which modulate differentially their activation, differentiation, and survival. The elucidation of these properties has led to the further use of EtxB as an agent capable of preventing the establishment of autoimmune diseases. The basis for these activities and their potential applicability to human therapies are discussed.

L22 ANSWER 17 OF 27 MEDLINE on STN DUPLICATE 11

1998435275. PubMed ID: 9762527. Effect of dehydroepiandrosterone (DHEA) on intestinal **mucosal** immunity in young adult and aging rats. Vargas J A; Vessey D A; Schmucker D L. (Cell Biology & Aging Section, Department of Veterans Affairs Medical Center, San Francisco, CA 94121, USA.) Experimental gerontology, (1998 Aug) Vol. 33, No. 5, pp. 499-505. Journal code: 0047061. ISSN: 0531-5565. Pub. country: ENGLAND: United Kingdom. Language: English.

AB The present study assesses the effectiveness of oral DHEA on the intestinal **mucosal** immune response in aging rats. Young adult (6 months) and aging (21 months) female rats received powdered rat chow with or without 0.2% DHEA for 23 days. The animals were immunized intraduodenally with either **cholera toxin** (**CTx**) or vehicle alone and boosted two weeks later. Seven days after boosting, serum, bile, small intestinal tissue, and liver were collected for analysis. Anti-**CTx** IgA antibody titers were measured in serum and bile and the concentration of anti-**CTx** antibody containing cells (ACCs) in the small intestinal lamina propria and liver were determined by quantitative immunohistochemistry. Intergroup comparisons indicated that there was only one significant difference in serum and none in bile anti-**CTx** IgA titers between **CTx** -immunized animals fed DHEA or the diet alone. Immunohistochemical analysis determined that the density and distribution patterns of ACCs within the lamina propria were unaffected by DHEA. Both DHEA-treated and control young immunized animals exhibited similar numbers of ACCs. Only 40% of the aging rats responded to intraduodenal immunization with **CTx**, as determined by the presence of ACCs in the intestine, regardless of the presence or absence of DHEA in the diet. These data suggest that DHEA in the diet does not enhance the intestinal **mucosal** immune response to intraduodenal **CTx** in either young adult or aging rats.

L22 ANSWER 18 OF 27 MEDLINE on STN DUPLICATE 12

96193838. PubMed ID: 8638712. Gastrin stimulation of histamine synthesis in enterochromaffin-like cells from rabbit fundic mucosa. Hollande F; Combettes S; Bali J P; Magous R. (Laboratorie de Biochimie des Membranes, Contrat Jeune Formation Institute National de la Sante et de la Recherche Medicale, Montpellier, France.) The American journal of physiology, (1996 Mar) Vol. 270, No. 3 Pt 1, pp. G463-9. Journal code: 0370511. ISSN: 0002-9513. Pub. country: United States. Language: English.

AB This work aimed to investigate the molecular role of gastrin in histamine synthesis in isolated rabbit fundic **mucosal** cells enriched in enterochromaffin-like (ECL) cells (37%). Gastrin stimulated histidine decarboxylase (HDC) activity by increasing the maximal velocity (Vmax) from 0.240 +/- 0.017 (basal value) to 0.332 +/- 0.012 pmol/mg protein/h and by decreasing the Michaelis-Menten constant value -Km; 73.90 +/- 2.2 vs. 93.42 +/- 4.32 microM (basal value)]. Pertussis toxin (PTX) (200 ng/ml) reduced the stimulation of HDC induced by 10 nM gastrin from 41.8 to 15.9%, whereas **cholera toxin** (**CTX**) (100 ng/ml) was without effect. Staurosporine and polymyxin B inhibited in a dose dependent manner the HDC activity stimulated by 10 nM gastrin.

Phorbol 12-myristate 13-acetate (PMA; 100 nM) decreased Vmax (0.558 +/- 0.021 pmol/ mg protein/h) but did not change the Km. Furthermore, cycloheximide (0.1-10 microM) inhibited the gastrin-induced stimulation of HDC activity, whereas actinomycin D (up to 10 microM) was without effect. Finally, incubation of cells with gastrin (10 microM) left the expression of HDC mRNA unchanged. We concluded that gastrin, acting through "gastrin/CCK-B type" receptors coupled to PTX-sensitive G protein, exerts a short-term regulation of histamine synthesis in gastric ECL cells by increasing both the affinity of HDC for L-histidine and the number of active enzyme molecules. This last event, related to protein kinase C activation, could be due to a translational or posttranslational mechanism.

L22 ANSWER 19 OF 27 SCISEARCH COPYRIGHT (c) 2006 The Thomson Corporation on STN

1996:220292 The Genuine Article (R) Number: UA050. Gastrin stimulation of histamine synthesis in enterochromaffin-like cells from rabbit fundic mucosa. Hollande F (Reprint); Combettes S; Bali J P; Magous R. FAC PHARM MONTPELLIER, BIOCHIM MEMBRANES LAB, CJF INSERM 9207, F-34060 MONTPELLIER, FRANCE. AMERICAN JOURNAL OF PHYSIOLOGY-GASTROINTESTINAL AND LIVER PHYSIOLOGY (MAR 1996) Vol. 33, No. 3, pp. G463-G469. ISSN: 0193-1857. Publisher: AMER PHYSIOLOGICAL SOC, 9650 ROCKVILLE PIKE, BETHESDA, MD 20814. Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB This work aimed to investigate the molecular role of gastrin in histamine synthesis in isolated rabbit fundic **mucosal** cells enriched in enterochromaffin-like (ECL) cells (37%). Gastrin stimulated histidine decarboxylase (HDC) activity by increasing the maximal velocity (V-max) from 0.240 +/- 0.017 (basal value) to 0.332 +/- 0.012 pmol . mg protein(-1). h(-1) and by decreasing the Michaelis-Menten constant value [K-m; 73.90 +/- 2.2 vs. 93.42 +/- 4.32 mu M (basal value)]. Pertussis toxin (PTX) (200 ng/ml) reduced the stimulation of HDC induced by 10 nM gastrin from 41.8 to 15.9%, whereas **cholera toxin** (**CTX**) (100 ng/ml) was without effect. Staurosporine and polymyxin B inhibited in a dose-dependent manner the HDC activity stimulated by 10 nM gastrin. Phorbol 12-myristate 13-acetate (PMA; 100 nM) decreased V-max (0.558 +/- 0.021 pmol . mg protein(-1). h(-1)) but did not change the K+. Furthermore, cycloheximide (0.1-10 mu M) inhibited the gastrin-induced stimulation of HDC activity, whereas actinomycin D (up to 10 mu M) was without effect. Finally, incubation of cells with gastrin (10 nM) left the expression of HDC mRNA unchanged. We concluded that gastrin, acting through ''gastrin/CCK-B type'' receptors coupled to PTX-sensitive G protein, exerts a short-term regulation of histamine synthesis in gastric ECL cells by increasing both the affinity of HDC for L-histidine and the number of active enzyme molecules. This last event, related to protein kinase C activation, could be due to a translational or posttranslational mechanism.

L22 ANSWER 20 OF 27 EMBASE COPYRIGHT (c) 2006 Elsevier B.V. All rights reserved on STN

96105706 EMBASE Document No.: 1996105706. Gastrin stimulation of histamine synthesis in enterochromaffin-like cells from rabbit fundic mucosa. Hollande F.; Combettes S.; Bali J.-P.; Magous R.. Lab. de Biochimie des Membranes, Faculte de Pharmacie, CJF INSERM 92-07, 15 Ave. Charles Flahault,34060 Montpellier Cedex, France. American Journal of Physiology - Gastrointestinal and Liver Physiology Vol. 270, No. 3 33-3, pp. G463-G469 1996.

ISSN: 0193-1857. CODEN: APGPDF

Pub. Country: United States. Language: English. Summary Language: English. Entered STN: 960430. Last Updated on STN: 960430

AB This work aimed to investigate the molecular role of gastrin in histamine synthesis in isolated rabbit fundic **mucosal** cells enriched in enterochromaffin-like (ECL) cells (37%). Gastrin stimulated histidine

decarboxylase (HDC) activity by increasing the maximal velocity (V(max)) from 0.240 ± 0.017 (basal value) to 0.332 ± 0.012 pmol · mg protein-1 · h- 1 and by decreasing the Michaelis-Menten constant value [K(m); 73.90 ± 2.2 vs. 93.42 ± 4.32 μM (basal value)]. Pertussis toxin (PTX) (200 ng/ml) reduced the stimulation of HDC induced by 10 nM gastrin from 41.8 to 15.9%, whereas **cholera toxin** (**CTX**) (100 ng/ml) was without effect. Staurosporine and polymyxin B inhibited in a dose-dependent manner the HDC activity stimulated by 10 nM gastrin. Phorbol 12-myristate 13-acetate (PMA; 100 nM) decreased V(max) (0.558 ± 0.021 pmol · mg protein-1 · h-1) but did not change the K(m). Furthermore, cycloheximide (0.1-10 μM) inhibited the gastrin- induced stimulation of HDC activity, whereas actinomycin D (up to 10 μM) was without effect. Finally, incubation of cells with gastrin (10 nM) left the expression of HDC mRNA unchanged. We concluded that gastrin, acting through 'gastrin/CCK-B type' receptors coupled to PTX-sensitive G protein, exerts a short-term regulation of histamine synthesis in gastric ECL cells by increasing both the affinity of HDC for L-histidine and the number of active enzyme molecules. This last event, related to protein kinase C activation, could be due to a translational or posttranslational mechanism.

L22 ANSWER 21 OF 27 SCISEARCH COPYRIGHT (c) 2006 The Thomson Corporation on STN

1995:546326 The Genuine Article (R) Number: RP396. INTESTINAL IMMUNIZATION OF MICE WITH ANTIGEN CONJUGATED TO ANTI-MHC CLASS-II ANTIBODIES. ESTRADA A (Reprint); MCDERMOTT M R; UNDERDOWN B J; SNIDER D P. MCMASTER UNIV, DEPT PATHOL, MOLEC VIROL & IMMUNOL PROGRAM, VACCINE DEV GRP, W HAMILTON, ON L8N 3Z5, CANADA. VACCINE (JUL 1995) Vol. 13, No. 10, pp. 901-907. ISSN: 0264-410X. Publisher: BUTTERWORTH-HEINEMANN LTD, LINACRE HOUSE JORDAN HILL, OXFORD, OXON, ENGLAND OX2 8DP. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB We have explored a new technique for immunization of the intestinal tract of mice, using protein antigens bound to antibodies with specificity for murine MHC class II molecules (MHC-II). Either of two protein antigens, hen avidin (AV) or hen egg lysozyme (HEL) were covalently conjugated to anti-MHC-II antibodies and the purified conjugates were given orally (p.o.) or by direct intraduodenal (i.d) injection into the intestinal lumen of mice. A secondary immunization p. o. with the same conjugate or with the non-conjugated antigen in the presence of **cholera toxin** (**CTX**) resulted in production of both intestinal secretory IgA and serum IgA antibody by those mice, In addition, serum IgG antibodies were produced Conjugates with appropriate MHC-II specificity targeted the antigen because they induced more IgA and IgG antibody than conjugates with irrelevant antibody specificity or antigen alone, and because they induced antibody in mice that were genetic low responders to antigen. The results indicate the feasibility of oral subunit type vaccines with antibody targeting technology.

L22 ANSWER 22 OF 27 MEDLINE on STN DUPLICATE 13

94292794. PubMed ID: 8021502. Production of IgE antibody and allergic sensitization of intestinal and peripheral tissues after oral immunization with protein Ag and **cholera toxin**. Snider D P; Marshall J S; Perdue M H; Liang H. (Department of Pathology, McMaster University, Hamilton, Ontario, Canada.) Journal of immunology (Baltimore, Md. : 1950), (1994 Jul 15) Vol. 153, No. 2, pp. 647-57. Journal code: 2985117R. ISSN: 0022-1767. Pub. country: United States. Language: English.

AB **Cholera toxin** (**CTX**) is a potent oral adjuvant for the induction of **mucosal** IgA Ab responses protein Ags. We examined the Ab responses and allergic sensitization of several strains of mice to protein Ags, administered orally with **CTX**. The mice made strong IgA and IgG1 serum Ab responses, but little IgG2a Ab to Ags such as hen egg lysozyme (HEL) and OVA. However, when given a

subsequent i.p. challenge with Ag alone, the same mice had immediate hypersensitivity reactions that included respiratory distress and death. Within 10 min of i.p. challenge, immunized mice had high levels of plasma histamine and extensive degranulation of mast cells in target tissues. These mice had detectable serum IgE Ab. Ag administered orally with the B subunit (CTB) of **CTX** did not sensitize mice. Intestinal tissues taken from these mice had Ag-specific ion-secretory responses in vitro, typical of intestinal anaphylaxis. Ag given s.c. without adjuvant could also sensitize for systemic and intestinal anaphylaxis. Sensitization with HEL given s.c. was dose dependent and correlated with a critical amount of HEL in the circulation. HEL was detected in the circulation after oral immunization, but **CTX** did not increase the uptake of HEL. Thus, oral immunization with a protein Ag in the presence of **CTX** can sensitize an animal for systemic and intestinal anaphylaxis. These results suggest a cautious approach to the use of **CTX** as an adjuvant in oral vaccines, and provide a new model to study immediate hypersensitivity reactions to intestinal Ag.

L22 ANSWER 23 OF 27 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN

1994:246123 Document No.: PREV199497259123. **Cholera toxin** (**CTX**) promotes IgE antibody and allergy during oral immunization. Snider, D. P.; Marshall, J. S.; Perdue, M. H.; Liang, H.. Dep. Pathology, MVIP, McMaster Univ., Hamilton, ON L8N 3Z5, Canada. FASEB Journal, (1994) Vol. 8, No. 4-5, pp. A282.
Meeting Info.: Experimental Biology 94, Parts I and II. Anaheim, California, USA. April 24-28, 1994.
CODEN: FAJOEC. ISSN: 0892-6638. Language: English.

L22 ANSWER 24 OF 27 SCISEARCH COPYRIGHT (c) 2006 The Thomson Corporation on STN DUPLICATE 14

1992:542876 The Genuine Article (R) Number: JM525. VACCINATION BY **CHOLERA-TOXIN** CONJUGATED TO A HERPES-SIMPLEX VIRUS TYPE 2 GLYCOPROTEIN D-PEPTIDE. DREW M D (Reprint); ESTRADACORREA A; UNDERDOWN B J; MCDERMOTT M R. MCMASTER UNIV, HLTH SCI CTR, DEPT PATHOL, MOLEC VIROL & IMMUNOL PROGRAM, ROOM 3N43A, 1200 MAIN ST W, HAMILTON L8N 3Z5, ONTARIO, CANADA. JOURNAL OF GENERAL VIROLOGY (SEP 1992) Vol. 73, Part 9, pp. 2357-2366. ISSN: 0022-1317. Publisher: SOC GENERAL MICROBIOLOGY, HARVEST HOUSE 62 LONDON ROAD, READING, BERKS, ENGLAND RG1 5AS. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Immunization of BALB/cJ mice with a peptide corresponding to residues 1 to 23 of glycoprotein D [gD(1-23)] from herpes simplex virus type 2 (HSV-2) elicits antibody responses which correlate with protection against lethal HSV-2 infection. In the present study, we examined the ability of **cholera toxin** (**CTX**) to act as an immunogenic carrier for gD(1-23). The number of gD(1-23) residues conjugated to **CTX** affected its binding to GM1 ganglioside and physiological toxicity, both of which are factors affecting oral immunogenicity. The antibody response elicited after intraperitoneal (i.p.) immunization with the **CTX**-gD(1-23) conjugate was protective against a lethal i.p. challenge with HSV-2. In other experiments, mice were immunized i.p. on day 0 and subsequent immunizations conducted on days 14 and 28 were administered either intragastrically or intravaginally (i.vag.). Intraperitoneal priming followed by either i.p. or intragastric boosting resulted in anti-HSV-2 antibodies in vaginal washings and in protection against a lethal i.vag. challenge with HSV-2. Intraperitoneal priming followed by i.vag. boosting did not elicit anti-HSV-2 antibodies in vaginal washings and did not protect mice against a lethal i.vag. challenge with HSV-2. These results suggest that **CTX** can act as a systemic and an oral delivery molecule for the covalently linked gD(1-23) peptide and that such conjugates can elicit protective immune responses against systemic and genital HSV-2 infection.

L22 ANSWER 25 OF 27 MEDLINE on STN DUPLICATE 15
92275665. PubMed ID: 1592437. Ageing compromises gastrointestinal **mucosal** immune response in the rhesus monkey. Taylor L D; Daniels C K; Schmucker D L. (Cell Biology and Aging, Veterans Administration Medical Center, University of California, San Francisco 94121.) Immunology, (1992 Apr) Vol. 75, No. 4, pp. 614-8. Journal code: 0374672. ISSN: 0019-2805. Pub. country: ENGLAND: United Kingdom. Language: English.

AB Most research on the effects of ageing on gut **mucosal** immunity has been performed using rodents. However, there are inherent difficulties in the extrapolation of rodent data to humans. This study was initiated to define age-related changes in the gastrointestinal (GI) **mucosal** immune response in non-human primates. Antibody responses were measured in young and old rhesus monkeys (Macaca mulatta) immunized intraduodenally with **cholera toxin** (**Ctx**)/cholera toxoid (Ctd). Antigen-specific immunoglobulin A (IgA) antibody levels were markedly lower while anti-**Ctx** IgG and IgM titres were higher in the intestinal lavage samples of old as compared to young animals. Total IgA concentrations in gut lavage were independent of age or immune status. Measurable titres of anti-**Ctx** IgA in the saliva of both age groups support the common **mucosal** immune hypothesis. Flow cytometric analysis was used to identify age-related shifts in the expression of cell surface antigens on peripheral blood lymphocytes. The relative number of both IgA+ and **Ctx**+ cells was dramatically reduced in the blood of old monkeys. Collectively, these data suggest that the GI **mucosal** immune response to **Ctx** is compromised in old rhesus macaques. The deficit in immune responsiveness, namely reduced anti-**Ctx** IgA antibody secretion into the intestinal lumen, may be a consequence of alterations in the process of maturation and homing of specific antibody-secreting B lymphocytes.

L22 ANSWER 26 OF 27 MEDLINE on STN DUPLICATE 16
91348854. PubMed ID: 1879933. Differential effect of aging on B-cell immune responses to **cholera toxin** in the inductive and effector sites of the **mucosal** immune system. Haq J A; Szewczuk M R. (Department of Microbiology and Immunology, Queen's University, Kingston, Ontario, Canada.) Infection and immunity, (1991 Sep) Vol. 59, No. 9, pp. 3094-100. Journal code: 0246127. ISSN: 0019-9567. Pub. country: United States. Language: English.

AB The age-associated primary immune response of B cells from the Peyer's patches (PP), the lamina propria (LP), the mesenteric lymph nodes (MLN), and the spleen of mice following oral immunization with **cholera toxin** (**CTx**) was investigated. The induction of immune responses was assessed in 4-, 11-, and 24-month-old, individual C57BL/6J male mice by determining the number and isotype of anti-**CTx** ELISPOT-forming cells (SFC) in the PP, LPL, MLN, and spleen and the titer and isotype of serum anti-**CTx** antibody. The data indicate a significant age-associated decline in immunoglobulin G (IgG) and IgA anti-**CTx** SFC in the LP B cells but only in IgA anti-**CTx** SFC in the PP. No decline was seen in the anti-**CTx** SFC response in the MLN and spleen. Peroral immunization of mice with **CTx** resulted in a serum anti-**CTx** antibody response which was predominantly of the IgG class in all three age groups of mice tested. There was no age-associated decline in anti-**CTx** IgM, IgG, or IgA titers in serum. Isoelectric focusing and affinity immunoblotting revealed several distinct new antibody clonotypes in the immune serum of old mice following oral immunization with **CTx**. The results indicate a loss of immune responsiveness to **CTx** following oral immunization in senescent PP and LP B cells. The MLN and spleen B-cell responses were found to be refractory to the loss of immune function with aging. These findings suggest a differential effect of aging in the inductive and effector sites of the **mucosal** immune system, and the loss of antigen-specific IgA responses at **mucosal** sites may

have adverse effects on the host's defense against potential pathogens.

L22 ANSWER 27 OF 27 MEDLINE on STN DUPLICATE 17
89006826. PubMed ID: 3169843. **Mucosal** immune response to **cholera toxin** in ageing rats. I. Antibody and antibody-containing cell response. Schmucker D L; Daniels C K; Wang R K; Smith K. (Cell Biology and Aging Section, Veterans Administration Medical Center, San Francisco, CA 94121.) Immunology, (1988 Aug) Vol. 64, No. 4, pp. 691-5. Journal code: 0374672. ISSN: 0019-2805. Pub. country: ENGLAND: United Kingdom. Language: English.

AB Although ageing is accompanied by systemic immunodeficiencies, the status of the **mucosal** immune system in the elderly remains unresolved. The gastrointestinal **mucosal** immune response was evaluated in young, mature and old male rats subjected to intra-intestinal immunization with **cholera toxin** (**CTx**). Five days following secondary immunization, the alpha-**CTx**-IgA titre in the bile of immunized rats was markedly reduced, i.e. the values measured in young rats were approximately five-fold higher than those of old animals. alpha-**CTx**-IgA levels in non-immunized rats were negligible and age-related shifts in other antibody titres (alpha-**CTx** IgG and IgM) were not significant. The antibody response to **CTx** was not reflected in the total IgA content of the samples. The number of alpha-**CTx** antibody-containing cells (ACCs) in the small intestinal lamina propria was significantly reduced in old immunized rats in comparison with the young or mature animals. These data suggest that ageing compromises both non-immune cell (antibody transport by hepatocytes) and immune cell (number of ACCs in the gut wall) functions in response to **cholera toxin** immunization in this animal model.

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FILE 'MEDLINE, EMBASE, BIOSIS, SCISEARCH, CAPLUS' ENTERED AT 08:37:25 ON 03 MAY 2006

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L1       294840 S ALLERGY
L2            2 S L1 AND ETX
L3            2 DUP REMOVE L2 (0 DUPLICATES REMOVED)
L4           14 S L1 AND CTX
L5           13 DUP REMOVE L4 (1 DUPLICATE REMOVED)
L6            1 S L1 AND ETXB
L7            1 S L1 AND CTXB
L8         9729 S ENTEROTOXIN B
L9          141 S L8 AND ALLERGEN
L10          60 DUP REMOVE L9 (81 DUPLICATES REMOVED)
L11          12 S L10 AND TREATMENT
L12          12 DUP REMOVE L11 (0 DUPLICATES REMOVED)
L13           3 S L10 AND MUCOSAL
L14           3 DUP REMOVE L13 (0 DUPLICATES REMOVED)
L15       48188 S CHOLERA TOXIN
L16        1970 S L15 AND CTX
L17           2 S L16 AND ALLERGY
L18           1 DUP REMOVE L17 (1 DUPLICATE REMOVED)
L19           0 S L16 AND ALLERGEN
L20         161 S L16 AND ANTIGEN
L21          75 S L16 AND MUCOSAL
L22          27 DUP REMOVE L21 (48 DUPLICATES REMOVED)
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L23         377 L15 AND CTXB
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L24 13 L23 AND COMBINATION

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L25 ANSWER 1 OF 5 EMBASE COPYRIGHT (c) 2006 Elsevier B.V. All rights reserved on STN
2003367900 EMBASE Expression and characterization of uropathogenic Escherichia coli adhesin protein linked to **cholera toxin** A2B subunits in Escherichia coli TB1. Lee Y.-H.; Ryu D.-K.; Kim B.-O.; Pyo S.. S. Pyo, Division of Immunopharmacology, College of Pharmacy, SungKyunKwan University, Suwon, Kyunggi-do 440-746, Korea, Republic of. snpyo@skku.ac.kr. Journal of Microbiology and Biotechnology Vol. 13, No. 4, pp. 552-559 2003.
Refs: 32.
ISSN: 1017-7825. CODEN: JOMBES
Pub. Country: Korea, Republic of. Language: English. Summary Language: English.
Entered STN: 20030925. Last Updated on STN: 20030925
AB The FimH subunit of type 1-fimbriated Escherichia coli (E. coli) has been determined as a major cause for urinary tract infections. Thus, to produce a possible vaccine antigen against urinary tract infections, the fimH gene was genetically coupled to the ctxa2b gene and cloned into a pMAL-p2E expression vector. The chimeric construction of pMALfimH/ctxa2b was then transformed into E. coli K-12 TB1 and its nucleotide sequence was verified. A fusion protein, based on fusing adhesin to the **cholera toxin** subunit A2B (CTXA2B), was induced with 0.01 mM isopropyl-β-D-thiogalactoside (IPTG) for 4 h at 37°C to yield a soluble fusion protein. The fusion protein was then purified by affinity chromatography. The expressed fusion protein was confirmed by SDS-PAGE and Western blotting using antibodies to the maltose binding protein (MBP) or the **cholera toxin** subunit B (**CTXB**), plus the N-terminal amino acid sequence was also analyzed. The orderly-assembled fusion protein was confirmed by a modified G(MI)-ganglioside ELISA, using antibodies to adhesin. The results indicated that the purified fusion protein was an adhesin/CTXA2B protein containing E. coli adhesin and the G(MI)-ganglioside binding activity of **CTXB**. Accordingly, this adhesin/CTXA2B protein may be a potential antigen for oral immunization against uropathogenic E. coli.

L25 ANSWER 2 OF 5 MEDLINE on STN DUPLICATE 1
2002294632. PubMed ID: 12034098. Comparison of mucosal and systemic humoral immune responses after transcutaneous and oral immunization strategies. John Manohar; Bridges Emily A; Miller Andy O; Calderwood Stephen B; Ryan Edward T. (Tropical & Geographic Medicine Center, Division of Infectious Diseases, Jackson 504, Massachusetts General Hospital, 55 Fruit Street, Boston, MA 02114, USA.) Vaccine, (2002 Jun 21) Vol. 20, No. 21-22, pp. 2720-6. Journal code: 8406899. ISSN: 0264-410X. Pub. country: England: United Kingdom. Language: English.
AB In order to compare the ability of transcutaneous and oral immunization strategies to induce mucosal and systemic immune responses, we inoculated mice transcutaneously with **cholera toxin** (CT) or the non-toxic B subunit of **cholera toxin** (**CtxB**), or orally with Peru2(pETR1), an attenuated vaccine strain of Vibrio cholerae expressing **CtxB**. In addition, we also evaluated dual immunization regimens (oral inoculation with transcutaneous boosting, and transcutaneous immunization with oral boosting) in an attempt to optimize induction of both mucosal and systemic immune responses. We found that transcutaneous immunization with purified **CtxB** or CT induces

much more prominent systemic IgG anti-**CtxB** responses than does oral inoculation with a vaccine vector strain of V. cholerae expressing **CtxB**. In comparison, anti-**CtxB** IgA in serum, stool and bile were comparable in mice either transcutaneously or orally immunized. Overall, the most prominent systemic and mucosal anti-**CtxB** responses occurred in mice that were orally primed with Peru2(pETR1) and transcutaneously boosted with CT. Our results suggest that **combination** oral and transcutaneous immunization strategies may most prominently induce both mucosal and systemic humoral responses.

L25 ANSWER 3 OF 5 EMBASE COPYRIGHT (c) 2006 Elsevier B.V. All rights reserved on STN

2001101614 EMBASE Peroral immunization with Helicobacter pylori adhesin protein genetically linked to **cholera toxin** A2B subunits. Kim B.O.; Shin S.S.; Yoo Y.H.; Pyo S.. Dr. S. Pyo, School of Pharmacy, Sung Kyun Kwan University, Suwon, 440-746 Kyunggi-Do, Korea, Republic of. snpyo@skku.ac.kr. Clinical Science Vol. 100, No. 3, pp. 291-298 2001.
Refs: 41.
ISSN: 0143-5221. CODEN: CSCIAE
Pub. Country: United Kingdom. Language: English. Summary Language: English.
Entered STN: 20010329. Last Updated on STN: 20010329

AB Helicobacter pylori is a major cause of gastric-associated diseases. To evaluate the efficacy of a possible vaccine antigen against H. pylori infection, the chimaeric construct adhesin-CTXA2B, derived from H. pylori adhesin genetically coupled to **cholera toxin** (CTX) subunits A2 and B (CTXA2B), was expressed in Escherichia coli as an insoluble recombinant chimaeric protein. The protein was then purified by denaturation, renaturation and size-exclusion chromatography. The composition of purified adhesin-CTXA2B was verified by SDS/PAGE and Western blotting with antibodies to antigenic components of adhesin and **CTXB**, and confirmed as a chimaeric protein with G(MI)-ganglioside binding activity and adhesin epitopes by a G(MI)-ELISA developed using antibodies to adhesin. Oral immunization of mice with adhesin-CTXA2B induced higher levels of mucosal IgA and serum IgG antibodies to H. pylori adhesin and to **CTXB** than in mice immunized with adhesin or CTXA2B alone. Adhesin-CTXA2B was also demonstrated to be a potential protective antigen in a mouse model of H. pylori infection. The immunization of mice with adhesin-CTXA2B protected 62.5% of mice infected with H. pylori SSI strain, whereas adhesin immunization was not able to confer protection to mice. This protection may be correlated with high levels of mucosal IgA and serum IgG antibodies against H. pylori adhesin. Taken together, the results indicate that the genetically linked CTXA2B acts as a useful mucosal adjuvant, and that the adhesin-CTXA2B chimaeric protein could be a potential component in future H. pylori vaccine development.

L25 ANSWER 4 OF 5 CAPLUS COPYRIGHT 2006 ACS on STN

2002:420039 Document No. 137:230880 Efforts towards the development of oral cholera vaccines in India against the backdrop of global endeavours. Ghosh, Amit; Thungapathra, M.; Sharma, C.; Gupta, N.; Ghosh, R. K.; Mukhopadhyay, A.; Kole, H.; Nair, G. B. (Institute of Microbial Technology, Chandigarh, 160 036, India). Diarrhoeal Diseases: Research Perspectives, [Lectures delivered at the Symposium on "New Perspectives of Research in Cholera and Diarrhoeal Diseases"], New Delhi, India, Mar. 18, 1998, Meeting Date 1998, 1-16. Editor(s): Rao, N. Appaji; Ganguly, N. K. Indian National Science Academy: New Delhi, India. ISBN: 81-7319-343-6 (English) 2000. CODEN: 69CQUD.

AB A review. According to the World Health Organization more than 70 million people died of infectious diseases in 1996. One of the infectious diseases which continues to cause global concern is cholera. Though it can be controlled by improved sanitation, this goal is not attainable in

most countries of the world. The development of an effective vaccine therefore, still remains the best solution As parenteral vaccines developed during the last 100 yr were found to be ineffective, in recent years two different approaches have been pursued for developing oral vaccines. While the first approach is based on the observation that a cholera patient develops both anti-toxic and anti-bacterial immunity, the rationale for the second approach is that convalescents develop lasting immunity against a fresh attack. Using the first approach, a **combination** vaccine comprising killed whole cells and the B subunit of **cholera toxin**, was developed. The outcome of the second approach was the development of ctxA-B+ strains of Vibrio cholerae (strains unable to synthesize the catalytic A subunit of the **cholera toxin**), which could mimic infection derived immunity in the host, when administered orally. However, all such strains were found to be reactogenic. A critical anal. of the available data indicated to us that the starting strain dets. the reactogenicity of the final construct. Hence a strain with requisite properties -completely non-reactogenic, devoid of all toxin genes and a good colonizer, was obtained after screening hundreds of isolates. The **ctxB** gene with its own up and downstream regulatory sequences, was then introduced into the chromosome of this strain at a specific locus. The recombinant strain designated VA1.3, was found to be completely non-reactogenic and 100% protective in animal studies. It was also found to be completely safe in toxicity studies conducted at the Post-Graduate Institute of Medical Education and Research, Chandigarh, under the guidance of Prof. N.K. Ganguly. The vaccine is now undergoing phase I trial. Approaches somewhat similar to the aforementioned ones have also been pursued by other groups in India. Thus while, Dr. B.S. Srivastava and his group (Central Drug Research Institute, Lucknow) have developed a potential subunit vaccine which looks promising, Dr. J. Das and coworkers (Indian Institute of Chemical Biol., Calcutta) have developed a plasmid based recombinant oral vaccine which has produced good results in animal studies.

L25 ANSWER 5 OF 5 MEDLINE on STN DUPLICATE 2

1998062149. PubMed ID: 9400977. Salmonella typhimurium aroA recombinants and immune-stimulating complexes as vaccine candidates for feline immunodeficiency virus. Tijhaar E J; Huisman W; Huisman R C; Siebelink K H; Karlas J A; de Ronde A; van Herwijnen R; Mooi F R; Osterhaus A D. (National Institute of Public Health and the Environment, Bilthoven, The Netherlands.) The Journal of general virology, (1997 Dec) Vol. 78 (Pt 12), pp. 3265-75. Journal code: 0077340. ISSN: 0022-1317. Pub. country: ENGLAND: United Kingdom. Language: English.

AB Two experimental feline immunodeficiency virus (FIV) vaccines were tested, either alone or in **combination**, in four groups of cats (A-D). One vaccine (SL3261-FIV) was composed of live attenuated Salmonella typhimurium aroA (SL3261) strains expressing the capsid (Gag) and part of the envelope (Env) proteins of FIV. The other was composed of FIV Gag and Env proteins incorporated into immune-stimulating complexes (iscom-FIV). Cats of group A were immunized four times with SL3261-FIV. Cats of group B were immunized twice with SL3261-FIV and then twice with iscom-FIV. Cats of group C were immunized twice with SL3261 expressing the B subunit of **cholera toxin** (SL3261-**CtxB**) and then twice with iscom-FIV. Cats of group D, which served as negative controls, were immunized twice with SL3261-**CtxB** and then twice with iscom into which the Gag and Env proteins of simian immunodeficiency virus (SIV) had been incorporated (iscom-SIV). Two weeks after the last immunization, all cats were challenged with FIV. At this time, cats immunized with iscom-FIV (groups B and C) showed strong plasma antibody responses to Gag and Env, whilst these responses were weak or undetectable in the cats immunized four times with SL3261-FIV (group A). Seven weeks after FIV challenge, Env-specific antibody responses had increased considerably in cats of all groups except group A. The mean virus loads in the cats of

this group proved to be lower than those of the other groups at all time points, indicating partial protection.

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L26 0 L23 AND ALLERGEN

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L28 ANSWER 1 OF 8 CAPLUS COPYRIGHT 2006 ACS on STN
2003:6139 Document No. 138:68275 Mutant forms of enterotoxin (EtxB) and **cholera toxin** (**CtxB**), and their therapeutic uses as target site-specific carriers. Hirst, Timothy Raymond (University of Bristol, UK). PCT Int. Appl. WO 2003000899 A1 20030103, 84 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2002-GB2829 20020620. PRIORITY: GB 2001-15382 20010622.
AB The present invention describes the use of a mutant form of enterotoxin subunit B (EtxB) or **cholera toxin** subunit B (**CtxB**) to deliver an agent to a target cell wherein the mutant has GM-1 binding activity, and a reduced immunogenic and immunomodulatory activity relative to the wild type form of EtxB or **CtxB**. Specifically, the mutant **CtxB** with His to Ala substitution at position 57 is severely defective as an immunomodulator, and the holotoxin exhibits ablated toxicity, and retains the ability to bind with high affinity to GM-1. The invention further discloses that EtxB or an EtxB(H57A) are able to act as trafficking mols. that facilitates delivery of exogenous epitopes into the endogenous pathway of class I antigen processing and presentation.

L28 ANSWER 2 OF 8 CAPLUS COPYRIGHT 2006 ACS on STN
2004:524595 Document No. 141:241687 Induction of a systemic IgG and secretory IgA responses in mice by peroral immunization with uropathogenic Escherichia coli adhesin protein coupled to **cholera toxin** A2B subunits. Lee, Yong-Hwa; Kim, Byung-Oh; Rhee, Dong-Kwon; Pyo, Suhkneung (Division of Immunopharmacology, College of Pharmacy, SungKyunKwan University, Suwon, Kyunggi-do, 440-746, S. Korea). Journal of Applied Pharmacology, 11(3), 157-162 (English) 2003. CODEN: JOAPA6. ISSN: 1225-6110. Publisher: Korean Society of Applied Pharmacology.
AB The generation of secretory IgA antibodies (Abs) for specific immune protection of mucosal surfaces depends on stimulation of the mucosal immune system, but this is not effectively achieved by parenteral or even oral administration of most soluble antigens. Thus, to produce a possible vaccine antigen against urinary tract infections, the uropathogenic E. coli (UPEC) adhesin was genetically coupled to the ctxa2b gene and cloned into a pMAL-p2E expression vector. The chimeric construction of pMALfimHlctxa2b was then transformed into E. coli K-12 TB1 and its nucleotide sequence was verified. The chimeric protein was then purified by applying the affinity chromatog. The purified chimeric protein was

confirmed by SDS-PAGE and western blotting. The orderly-assembled chimeric protein was confirmed by a modified GMI ganglioside ELISA using antibodies to adhesin. The results indicate that the purified chimeric protein was an adhesin/CTXa2B protein containing UPEC adhesin and the GMI ganglioside binding activity of **CTXB**. The study also demonstrates that peroral administration of this chimeric immunogen in mice elicited high levels of secretory IgA and serum IgG Abs to the UPEC adhesin. Apparently, the genetically linked CTXA2B acts as a useful mucosal adjuvant, and the adhesin/CTXA2B chimeric protein might be a potential antigen for oral immunization against UPEC.

L28 ANSWER 3 OF 8 MEDLINE on STN DUPLICATE 1

2002150343. PubMed ID: 11882700. Contribution of the ADP-ribosylating and receptor-binding properties of cholera-like enterotoxins in modulating cytokine secretion by human intestinal epithelial cells. Soriani Marco; Bailey Lorna; Hirst Timothy R. (Department of Pathology and Microbiology, University of Bristol, Bristol BS8 1TD, UK.) Microbiology (Reading, England), (2002 Mar) Vol. 148, No. Pt 3, pp. 667-76. Journal code: 9430468. ISSN: 1350-0872. Pub. country: England: United Kingdom. Language: English.

AB When epithelial cells first encounter **cholera toxin** (Ctx) produced by Vibrio cholerae they secrete not only chloride ions responsible for causing diarrhoea, but also a number of cytokines that may contribute to the toxin's potent immunomodulatory properties. Much less is known about the ability of the heat-labile enterotoxin of Escherichia coli (Etx), a close homologue of Ctx, to elicit cytokine secretion by epithelial cells. This study shows that **treatment** of human intestinal epithelial T84 cells with Etx induces expression of IL-6, IL-10, IL-1R antagonist, as well as IL-1alpha and IL-1beta and low levels of IL-8. Such induction was totally dependent on the intrinsic ADP-ribosylating activity of the toxin A-subunit, and could be mimicked by cAMP-elevating agents, such as forskolin and dibutyryl cAMP. By comparison, neither an enzymically inactive mutant of Etx nor EtxB was able to induce cytokine secretion. The behaviour of Ctx and **CtxB** was very similar to that of Etx and EtxB, respectively. The spectrum of cytokines released by Etx and Ctx indicates that the toxins may create a local microenvironment that strongly biases the immune response towards an anti-inflammatory and a polarized Th2 response.

L28 ANSWER 4 OF 8 CAPLUS COPYRIGHT 2006 ACS on STN

2003:84556 Document No. 138:336065 Preparation and immunogenicity study of HspA-**CtxB** fusion protein to Helicobacter pylori in mice. Li, Mingfeng; Liu, Xinmei; Ling, Zhen; He, Zhiyong; Shen, Xudong; Sun, Jianxin; Wu, Xiangfu (Department of Gastroenterology, 455 Hospital of PLA, Shanghai, 200052, Peop. Rep. China). Zhonghua Weishengwuxue He Mianyixue Zazhi, 22(4), 398-402 (Chinese) 2002. CODEN: ZWMZDP. ISSN: 0254-5101. Publisher: Weishenbu Beijing Shengwu Zhipin Yanjiuso.

AB The immune response in mice fed orally with the conjugated antigen of HspA-**CtXB** (HCT) as well as the absorption of the antigen in the small intestine of mice was studied. LlkkA recombinant strain which could express bivalent antigen of HspA and **CtxB** subunit was constructed. HspA and hct gene was amplified by PCR. The DNA products of HspA and **CtxB** were inserted into a prokaryotic expression vector pET-22b(+) resp., and then transfected into E.coli strain BL-21 (DE3) to express HCT fusion protein. Hct gene was sequenced as 726 base pairs, the fusion protein encoded polypeptides of 242 amino acid residues, corresponding to calculated mol. masses(Mr) of 30 x 103. Western blot anal. of the recombinant protein HCT confirmed that it could he specifically recognized by the serum of H.pylori-infected patients. HspA and HCT labeled 125I were orally delivered into the stomach of mice resp., and the radioactivity of 125I in serum of each mouse was detected in different periods: 5 min, 10 min, 15 min, 30 min, 60 min and 90 min. Antibody response in animals immunized with recombinant HCT or HspA was determined by

ELISA. In the group of HCT, serum IgA and IgG were 2.98±0.35 and 4.38±0.56 resp.; fecal IgA was 2.89±0.68. In the HspA, serum IgA and IgG were 0.38±0.15 and 0.45±0.16 resp.; fecal IgA was 0.69±0.23. In normal control group, serum IgA and IgG were 0.06±0.02 and 0.09±0.06 resp.; fecal IgA 0.12±0.03. These results indicate that HCT can be immediately absorbed in a large amount from small intestine of mice, and high value of HspA-specific antibodies are produced by orally immunization with HCT. The recombinant fusion protein HCT can be used as an effective oral vaccine for prevention and **treatment** of infection of H.pylori.

L28 ANSWER 5 OF 8 EMBASE COPYRIGHT (c) 2006 Elsevier B.V. All rights reserved on STN DUPLICATE 2

2002410564 EMBASE In vitro expression of the **ctxB** toxin gene towards the development of a DNA vaccine against cholera. Syahril N.A.; Mariana N.S.; Sidhu H.S.; Rosli R.. R. Rosli, Faculty of Medicine/Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia. rozita@medic.upm.edu.my. Molecular Biology Today Vol. 3, No. 3, pp. 71-77 2002.
Refs: 23.
ISSN: 1468-5698. CODEN: MBTOC8
Pub. Country: United Kingdom. Language: English. Summary Language: English.
Entered STN: 20021202. Last Updated on STN: 20021202

AB The complete eradication of cholera is an unachievable goal because it is now firmly established that there are environmental reservoirs for Vibrio cholerae. Although there are effective **treatments** for this disease, they are expensive and impractical in times of epidemic. All these points lead to the fact that the development of a safe, cheap, and efficient vaccine is probably the best solution to the problem. A new generation of vaccine, termed DNA vaccine, would probably be a better alternative to the traditional vaccines. In this study, the focus is on the **ctxB**, the gene encoding the B subunit **cholera toxin** as a potential candidate for the DNA vaccine against cholera. The **ctxB** gene is required for the binding of the **Cholera Toxin** (CT) to the eukaryotic cell and facilitates the entry of the active toxin (CTXA) into the host cell which causes the profuse diarrheal symptom. The **ctxB** gene was cloned in pVax 1 (Invitrogen), and proven to be in the correct orientation. Subsequently, expression of the B subunit toxin in vitro, was successfully carried out using 10 and 20 ml of Effectene® (Qiagen) reagent with 0.4 mg pVax/**ctxB**, 90 hours post transfection in COS-7 cells. The results showed that the pentamer size of the **ctxB** (58 kDa) was expressed instead of its single monomer of 11.6 kDa. This means that the mechanism of the eukaryotic expression system in vitro was able to produce this end-product by successfully processing the binding of five single peptides of the **ctxB**. Further investigations involving this potential DNA vaccine against cholera is currently underway, including the production of antibody in animal models.

L28 ANSWER 6 OF 8 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN DUPLICATE 3

2001:290633 Document No.: PREV200100290633. A candidate oral vaccine to Helicobacter pylori: Fusion protein of HspA and **CtxB**. Li Ming-Feng; He Zhi-Yong; Ling Zheng; Wang Jiao-Yang; Sheng Xu-Dong; Yang Guan-Zhen; Wu Xiang-Fu [Reprint author]. Shanghai Institute of Biochemistry, Chinese Academy of Sciences, Shanghai, 200031, China. xfwu@sunm.shcnc.ac.cn. Shengwu Huaxue yu Shengwu Wuli Xuebao, (May, 2001) Vol. 33, No. 3, pp. 360-364. print.
ISSN: 0582-9879. Language: Chinese.

AB Heat-shock protein A subunit (HspA), an effective immunogen may stimulate the immunoresponse in human body against challenge of H. pylori. The B subunit of **cholera toxin** (**CtxB**) has been

proved to be a potent mucosal immunogen, act as an adjuvant for vaccine targeted for delivery to the mucosal-associated lymphoid tissue. A recombinant plasmid expressing bivalent antigen of HspA and **CtxB** subunit was constructed as follows. hspA and **ctxB** gene was amplified by PCR. The DNA products of hspA and **ctxB** were inserted in the prokaryotic expression vector pET-22b(+), respectively, and then the resulted recombinant plasmid expressing a fusion protein named HCT was transformed into the E. coli strain BL-21(DE3). hct gene was measured to be 708 base pairs long, and the fusion protein encoding a polypeptide of 236 amino acid residues, corresponded to a calculated molecular masses of 30 kD. Western blot analysis of the recombinant protein HCT confirmed that it could be specifically recognized by the serum of H. pylori-infected patients. HspA and HCT labelled 125I were orally administered into the stomach of mice, respectively, and the radioactivity of 125I in serum of each mouse was assayed at intervals: 15 min, 30 min, 60 min, 90 min and 120 min. The result indicated that there were high radioactivity counts in the groups of HCT than that of HspA ($P < 0.001$). This result suggests that the **CtxB** may enhance the volume of HspA absorbed from the intestine of mice, therefore the recombinant fusion protein HCT may be an effective oral vaccine for prevention and **treatment** against the infection of H. pylori.

L28 ANSWER 7 OF 8 MEDLINE on STN DUPLICATE 4

2001210820. PubMed ID: 11298654. **Cholera toxin** and Escherichia coli enterotoxin B-subunits inhibit macrophage-mediated antigen processing and presentation: evidence for antigen persistence in non-acidic recycling endosomal compartments. Millar D G; Hirst T R. (Department of Pathology and Microbiology, University of Bristol, School of Medical Sciences, Bristol BS8 1TD, UK.) Cellular microbiology, (2001 May) Vol. 3, No. 5, pp. 311-29. Journal code: 100883691. ISSN: 1462-5814. Pub. country: England: United Kingdom. Language: English.

AB **Cholera toxin** (Ctx) and the closely related Escherichia coli heat-labile enterotoxin (Etx) not only act as mediators of diarrhoeal disease but also exert potent immunomodulatory properties on mammalian immune systems. The toxins normally exert their diarrhoeagenic effects by initiating receptor-mediated uptake into vesicles that enter a retrograde trafficking pathway, circumventing degradative compartments and targeting them to the trans-Golgi network (TGN) and endoplasmic reticulum. Here, we examine whether receptor-mediated binding and cellular entry by the toxin B-subunits also lead to concomitant changes in uptake and trafficking of exogenous antigens that could contribute to the potent immunomodulatory properties of these toxins. **Treatment** of the macrophage (J774.2) cell line with Etx B-subunit (EtxB) resulted in EtxB transport to the TGN and also led to the formation of large, translucent, non-acidic, EtxB-devoid vacuoles. When exogenous antigens were added, EtxB-treated cells were found to be proficient in both internalization of ovalbumin (OVA) and phagocytosis of bacterial particles. However, the internalized OVA, instead of trafficking along a lysosome-directed endocytic pathway via acidified endosomes, persisted in a non-acidic, light-density compartment that was distinct from the translucent vacuoles. The rerouted OVA did not co-localize with the endosomal markers rab5 or rab11, nor with EtxB, but was retained in a transferrin receptor-positive compartment. The failure of OVA to enter the late endosomal/lysosomal compartments correlated with a striking inhibition of OVA peptide processing and presentation to OVA-responsive CD4+ T-cells. **CtxB** also modulated OVA trafficking and inhibited antigen presentation. These findings demonstrate that the B-subunits of Ctx and Etx alter the progression of exogenous antigens along the endocytic processing pathway, and prevent or delay efficient epitope presentation and T-cell stimulation. The formation of such 'antigen depots' could contribute to the immunomodulatory properties of these bacterial virulence determinants.

L28 ANSWER 8 OF 8 MEDLINE on STN

93014215. PubMed ID: 1399002. Fusion proteins containing the A2 domain of **cholera toxin** assemble with B polypeptides of **cholera toxin** to form immunoreactive and functional holotoxin-like chimeras. Jobling M G; Holmes R K. (Department of Microbiology, Uniformed Services University of the Health Sciences, Bethesda, Maryland 20814.) Infection and immunity, (1992 Nov) Vol. 60, No. 11, pp. 4915-24. Journal code: 0246127. ISSN: 0019-9567. Pub. country: United States. Language: English.

AB Cholera enterotoxin (CT) is produced by Vibrio cholerae and excreted into the culture medium as an extracellular protein. CT consists of one A polypeptide and five B polypeptides associated by noncovalent bonds, and CT-B interacts with CT-A primarily via the A2 domain. **Treatment** of CT with trypsin cleaves CT-A into A1 and A2 fragments that are linked by a disulfide bond. CT-B binds to ganglioside GM1, which functions as the plasma membrane receptor for CT, and the enzymatic activity of A1 causes the toxic effects of CT on target cells. We constructed translational fusions that joined foreign proteins via their carboxyl termini to the A2 domain of CT-A, and we studied the interactions of the fusion proteins with CT-B. The A2 domain was necessary and sufficient to enable bacterial alkaline phosphatase (BAP), maltose-binding protein (MBP) or beta-lactamase (BLA) to associate with CT-B to form stable, immunoreactive, holotoxin-like chimeras. Each holotoxin-like chimera was able to bind to ganglioside GM1. Holotoxin-like chimeras containing the BAP-A2 and BLA-A2 fusion proteins had BAP activity and BLA activity, respectively. We constructed BAP-A2 mutants with altered carboxyl-terminal sequences and tested their ability to assemble into holotoxin-like chimeras. Although the carboxyl-terminal QDEL sequence of the BAP-A2 fusion protein was not required for interaction with CT-B, most BAP-A2 mutants with altered carboxyl termini did not form holotoxin-like chimeras. When holotoxin-like chimeras containing BAP-A2, MBP-A2, or BLA-A2 were synthesized in V. cholerae, they were found predominantly in the periplasm. The toxin secretory apparatus of V. cholerae was not able, therefore, to translocate these holotoxin-like chimeras across the outer membrane.

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=> s l23 and vaccine
L29         174 L23 AND VACCINE

=> dup reomve l29
ENTER REMOVE, IDENTIFY, ONLY, OR (?):remove
'REOMVE' IS NOT VALID.  VALID FILE NAMES ARE 'MEDLINE, EMBASE, BIOSIS, SCISEARCH,
CAPLUS'
You have entered a file name of duplicates to keep that is not
referenced by any of the L#s specified for this DUPLICATE command.
The file names of duplicates that can be kept are listed above.
Please enter one of these file names.

=> dup remove l29
PROCESSING COMPLETED FOR L29
L30          71 DUP REMOVE L29 (103 DUPLICATES REMOVED)

=> d l30 1-71 cbib abs
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L30 ANSWER 1 OF 71 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN
2005:337771 Document No.: PREV200510123875. Intradermal immunization of mice with **cholera toxin** B-pneumococcal surface protein A fusion protein is protective against intraperitoneal challenge with Streptococcus pneumoniae. Areas, Ana Paula Mattos; Oliveira, Maria Leonor Sarno; Miyaji, Eliane Namie; Leite, Luciana Cezar Cerqueira; Ho, Paulo Lee [Reprint Author]. Inst Butantan, Ctr Biotechnol, Sao Paulo, Brazil. hoplee@butantan.gov.br. Infection and Immunity, (JUN 2005) Vol. 73, No. 6, pp. 3810-3813.

CODEN: INFIBR. ISSN: 0019-9567. Language: English.

L30 ANSWER 2 OF 71 MEDLINE on STN

2005124457. PubMed ID: 15756423. Co-expression and immunity of Legionella pneumophila mip gene and immunoadjuvant **ctxB** gene. Wang Tao; Chen Jian-Ping; Li Hong; Zhi Ke-Qian; Zhang Lei; Yang Chun-Lei; Tao Da-Chang. (School of Preclinical and Forensic Medicine of West China, Sichuan University, Chengdu, China.) Acta biochimica et biophysica Sinica, (2005 Mar) Vol. 37, No. 3, pp. 199-204. Journal code: 101206716. ISSN: 1672-9145. Pub. country: China. Language: English.

AB The mip gene of Legionella pneumophila and the **ctxB** gene of Vibrio cholerae were amplified by PCR respectively. The amplified cDNA was ligated to the pcDNA3.1(+) vector. The recombinant plasmids pcDNA3.1-mip and pcDNA3.1-**ctxB** were identified by restriction analysis and PCR, and further confirmed by sequencing analysis. NIH3T3 cells were transfected with pcDNA3.1-mip and pcDNA3.1-**ctxB** according to the Lipofection method. Transient and stable products of the co-expression of the mip gene and **ctxB** gene were detected by immunofluorescence and Western blotting. The results showed that NIH3T3 cells were successfully transfected, and that the transiently and stably co-expressed products can be detected in the transfected cells. To detect the humoral and cellular immune response in immunized mice induced by the co-mmunization of the mip and **ctxB** genes, female BALB/c mice were immunized intramuscularly with pcDNA3.1-mip and pcDNA3.1-**ctxB** . The results showed that the specific antibody titer and the cytotoxic T-lymphocyte response for pcDNA3.1-mip immunization and co-immunization were increased compared with that of pcDNA3.1(+) immunization. Furthermore, the specific antibody titer and cytotoxic T-lymphocyte response for co-immunization were increased compared with that of pcDNA3.1-mip immunization. Statistical analysis using one-way analysis of variance (ANOVA) showed that there was a significant difference between the groups ($P<0.01$). The results indicated that the **ctxB** gene enhanced the humoral and cellular immune response to the mip gene immunization. These findings provide experimental evidence to support the development of the L. pneumophila DNA **vaccine**.

L30 ANSWER 3 OF 71 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN DUPLICATE 1

2005:399147 Document No.: PREV200510183119. Expression of **cholera toxin** B subunit in Saccharomyces cerevisiae. Arzanlou, Mohsen; Rezaee, Abbas [Reprint Author]; Shahrokhi, Nader; Hossini, Ahmad Zavaran; Yasuda, Yoko; Tochikubo, Kunio; Ahangarzadeh Rezaee, Mohammad. 106,Immam Zaman Alley,95 Sq, Tehran 16456, Iran. abbasrezaee@yahoo.com. Annals of Microbiology, (2005) Vol. 55, No. 2, pp. 145-150. ISSN: 1590-4261. Language: English.

AB **Cholera toxin**, secreted by Vibrio cholerae, consists of A and B subunits. **Cholera toxin** B subunit (CTB) is used in many scientific researches. It has already been expressed in several bacterial and plant systems. In order to express CTB protein in Saccharomyces cerevisiae, the expression plasmid pCTB83 was constructed by inserting **ctxB** gene in pYES2 shuttle vector. The new construct was transferred into S. cerevisiae cells and the **ctxB** gene was induced with 2% galactose. SDS-PAGE analysis showed the presence of CTB in yeast lysate and immunoblotting analysis of yeast total soluble protein indicated that the yeast-derived CTB protein was antigenically indistinguishable from bacterial CTB protein. Quantitative ELISA showed that the maximum amount of CTB protein expressed in yeast was approximately 1.8% of total soluble protein. CTB is a subunit **vaccine** candidate against cholera. Since the whole recombinant yeast has been introduced as a new **vaccine** formulation, expression of **ctxB** in S. cerevisiae may offer an effective and inexpensive strategy to protect people against cholera in high-risk areas.

L30 ANSWER 4 OF 71 CAPLUS COPYRIGHT 2006 ACS on STN
2004:899547 Document No. 141:330777 Strain of bacterium Escherichia coli KM-147 expressing Vibrio cholerae **ctxB** gene as a producer of B-subunit of **cholera toxin**. Smirnova, N. I.; Chekhovskaya, G. V.; Livanova, L. F.; Kobkova, I. M. (Federal'noe Gosudarstvennoe Uchrezhdenie Rossiiskii Nauchno-Issledovatel'skii Protivochumnyi Institut "Mikrob", Russia). Russ. RU 2238975 C1 20041027, No pp. given (Russian). CODEN: RUXXE7. APPLICATION: RU 2003-105594 20030226.

AB The invention relates to microbiol. and genetic engineering, and particularly to constructing the strain of Escherichia coli as a producer of choleraic toxin. The invention provides preparing the non-pathogenic for human and animals strain of E. coli with high production of basic protective antigen Vibrio cholerae (**cholera toxin** B-subunit). The strain is generated on the basis of avirulent strain E. coli KM-147. Recombinant plasmid vector pIEM3 (KmrTcr) with the cloned gene of **cholera toxin** (**ctxB**) was inserted into E. coli KM-147 cells by conjugative method. The proposed strain is deposited in the State collection of pathogenic microorganisms RosNIPCHI Mikrob. The invention can be used for creating diagnostic test-system for revealing toxicogenic clones of V. cholerae and E. coli and for constructing live **vaccine** strains against diarrheal diseases caused by pathogenic strains of V. cholerae and E. coli.

L30 ANSWER 5 OF 71 CAPLUS COPYRIGHT 2006 ACS on STN
2004:769607 Document No. 141:348812 Strain of bacterium Vibrio cholerae KM-168 as base for construction of live **vaccine** against diarrhea disease caused by pathogenic strains of Vibrio cholerae O139. Osin, A. V.; Livanova, L. F.; Kobkova, I. M.; Eroshenko, G. A.; Smirnova, N. I. (Federal'noe Gosudarstvennoe Uchrezhdenie Rossiiskii Nauchno-Issledovatel'skii Protivochumnyi Institut "Mikrob", Russia). Russ. RU 2236455 C1 20040920, No pp. given (Russian). CODEN: RUXXE7. APPLICATION: RU 2003-131557 20031027.

AB The Vibrio cholerae strain O130 is obtained by mutagenesis and the following insertion of recombinant plasmid pIEM3 (KmrTcr) with cloned gene of **cholera toxin** (**ctxB**) β-subunit. The strain has no hemolytic trait, it does not comprise the main virulence genes and is avirulent for laboratory animals. The strain exhibits high and stable level in production of **cholera toxin** B-subunit (it produces 2.5-3 mcg/mL of B-subunit XT in medium). The protective antigens of cholera pathogens of serum group O139 - antigen O139 and polysaccharide capsule provide the formation of antibacterial and antitoxic immunity to cholera.

L30 ANSWER 6 OF 71 SCISEARCH COPYRIGHT (c) 2006 The Thomson Corporation on STN
2004:868461 The Genuine Article (R) Number: 856NT. Study of expression in vitro and immunogenicity of mip/**ctxB** fusion gene of Legionella pneumophila. Wang T; Chen J P (Reprint); Zhi K Q; Tao D C; Yang C L; Zhang L. Sichuan Univ, W China Med Ctr, Dept Parasitol, Chengdu 610041, Peoples R China (Reprint); Xian Jiaotong Univ, Coll Stomatol, Dept Oral & Maxillofacial Surg, Xian 710004, Peoples R China; Sichuan Univ, Life Sci Coll, Dept Med Cell Biol, Chengdu 610041, Peoples R China; Dali Coll, Dept Microbiol, Dali 671000, Peoples R China. jpchen007@163.com. PROGRESS IN BIOCHEMISTRY AND BIOPHYSICS (SEP 2004) Vol. 31, No. 9, pp. 818-823. ISSN: 1000-3282. Publisher: SCIENCE CHINA PRESS, 16 DONGHUANGCHENGGEN NORTH ST, BEIJING 100717, PEOPLES R CHINA. Language: Chinese.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Mip gene of Legionella pneumophila and **ctxB** gene of Vibrio cholerae were PCR amplified respectively. The amplified fusion DNA was ligated to pcDNA3. 1 (+) vector. The recombinant plasmid, which was named pcDNA3. 1-mip/**ctxB**, was identified by restriction analysis, PCR and further confirmed by sequence analysis. NIH3T3 cell was transfected

by recombinant plasmid pcDNA3. 1-mip or pcDNA3. 1-mip/**ctxB** with Lipofection strategy. Transient and stable products of mip/**ctxB** fusion gene was detected by immunofluorescence and Western blot. The results showed that NIH3T3 cell was transfected successfully and stable products can be detected in the transfected cell. To evaluate immunonicity of pcDNA3. 1-mip and pcDNA3. 1-mip/**ctxB**, BALB/c female mice were immunized intramuscularly with them and antigen specific antibodies, lymphocyte proliferative response, IFN-gamma production and cytotoxic T-lymphocyte response of immunized mice were detected. The results showed that immunogenicity of pcDNA3. 1-mip or pcDNA3. 1-mip/ **ctxB** immunized mice were higher than control and immunogenicity of peDNA3. 1-mip/**ctxB** immunized mice were higher than pcDNA3. 1-mip immuneized mice. Statistic analysis by one way ANOVA showed that there was significantly difference between groups ($P < 0.01$). The results provide experimental proof to study of mip/**ctxB** fusion gene DNA **vaccine**.

L30 ANSWER 7 OF 71 SCISEARCH COPYRIGHT (c) 2006 The Thomson Corporation on STN DUPLICATE 2

2004:507282 The Genuine Article (R) Number: 823PV. Synthesis and assembly of a **cholera toxin** B subunit SHIV 89.6p Tat fusion protein in transgenic potato. Kim T G; Ruprecht R; Langridge W H R (Reprint). Loma Linda Univ, Dept Biochem & Microbiol, Ctr Mol Biol & Gene Therapy, Sch Med, Loma Linda, CA 92350 USA (Reprint); Harvard Univ, Sch Med, Dana Farber Canc Inst, Boston, MA 02115 USA. PROTEIN EXPRESSION AND PURIFICATION (JUN 2004) Vol. 35, No. 2, pp. 313-319. ISSN: 1046-5928. Publisher: ACADEMIC PRESS INC ELSEVIER SCIENCE, 525 B ST, STE 1900, SAN DIEGO, CA 92101-4495 USA. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB A cDNA encoding the simian-human immunodeficiency virus (SHIV 89.6p) Tat regulatory element protein was fused to the c-terminus of the **cholera toxin** B subunit gene (**ctxB**-tat) and introduced into Solarium tuberosum cells by Agrobacterium tumefaciens-mediated transformation methods. The fusion gene was detected in the genomic DNA of transformed potato leaf cells by PCR DNA amplification. Synthesis and assembly of the CTB-Tat fusion protein into oligomeric structures of pentamer size was detected in transformed tuber extracts by immunoblot analysis. The binding of CTB-Tat fusion protein pentamers to intestinal epithelial cell membrane glycolipid receptors was quantified by G(M1)-ganglioside enzyme-linked immunosorbent assay (G(M1)-ELISA). Based on the ELISA results, CTB-Tat fusion protein made up about 0.005-0.007% of total soluble tuber protein or approximately 4.6 mg per 100 g potato tuber tissue. The synthesis and assembly of CTB-Tat monomers into biologically active oligomers in transformed potato tuber tissues demonstrates the feasibility of using viral pathogen antigens synthesized in edible plants for mucosal immunization against HIV-1 infection.

L30 ANSWER 8 OF 71 MEDLINE on STN DUPLICATE 3

2004457710. PubMed ID: 15358234. Expression and characterization of **cholera toxin** B-pneumococcal surface adhesin A fusion protein in Escherichia coli: ability of CTB-PsaA to induce humoral immune response in mice. Areas Ana Paula Mattos; Oliveira Maria Leonor Sarno; Miyaji Eliane Namie; Leite Luciana Cezar Cerqueira; Aires Karina Araujo; Dias Waldely Oliveira; Ho Paulo Lee. (Centro de Biotecnologia, Instituto Butantan, Sao Paulo, Brazil.) Biochemical and biophysical research communications, (2004 Aug 13) Vol. 321, No. 1, pp. 192-6. Journal code: 0372516. ISSN: 0006-291X. Pub. country: United States. Language: English.

AB **Cholera toxin** B subunit (CTB) is responsible for CT holotoxin binding to the cell and has been described as a mucosal adjuvant for **vaccines**. In this work, the **ctxB** gene was genetically fused to the psaA gene from Streptococcus pneumoniae, a surface protein involved in its colonization in the host that is also

considered a **vaccine** antigen candidate against this pathogen. The CTB-PsaA fusion protein was expressed in Escherichia coli, and the purified protein was used for intranasal immunization experiments in Balb/C mice. CTB-PsaA was able to induce both systemic and mucosal antibodies evaluated in serum, saliva, and in nasal and bronchial wash samples, showing that CTB-PsaA is a promising molecule to be investigated as S. pneumoniae **vaccine** antigen candidate.

L30 ANSWER 9 OF 71 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN
2004:292303 Document No.: PREV200400291785. Peroral Immunization of uropathogenic Escherichia coli Adhesin protein Linked to **Cholera Toxin** A2B subunits. Lee, Yonghwa [Reprint Author]; Rhee, DongKwon; Pyo, Suhkneung. College of Pharmacy, Sungkyunkwan University, 300 Chunchun-dong Jangan-gu, Suwon, Kyunggi-do, 440-746, South Korea. snpyo@skku.ac.kr. FASEB Journal, (2004) Vol. 18, No. 4-5, pp. Abst. 558.21. http://www.fasebj.org/. e-file.
Meeting Info.: FASEB Meeting on Experimental Biology: Translating the Genome. Washington, District of Columbia, USA. April 17-21, 2004. FASEB.
ISSN: 0892-6638 (ISSN print). Language: English.

AB The FimH subunit of type 1-fimbriated uropathogenic Escherichia coli (UPEC) has been determined as a major cause for urinary tract infections. The chimaeric construct adhesin-LTXA2B derived from UPEC FimH adhesin genetically coupled to **cholera Toxin** (CTX) subunits A2 and B (CTXA2B) was expressed in E. coli as an soluble recombinant chimaeric protein. We have evaluated the efficacy of a possible **vaccine** antigen, FimH adhesin-CTXA2B chimaeric protein, against urinary tract infections. The protein was purified by osmotic shock and affinity chromatography. The composition of purified FimH adhesin-CTXA2B was verified by SDS/PAGE and Western blotting with antibodies to antigenic components of FimH adhesin and **CTXB**, and confirmed as a chimaeric protein with GM1 ganglioside binding activity and FimH adhesin epitopes by a GM1-ELISA developed using antibodies to FimH adhesin. Oral immunization of mice with FimH adhesin-CTXA2B induced higher level of mucosal IgA and serum IgG antibodies to FimH adhesin and to LTXB than in mice immunized with FimH adhesin or CTXA2B alone. FimH adhesin-CTXA2B was also demonstrated to be potential protective and therapeutic antigens in a mouse model infected with uropathogenic E. coli J96. Taken together, the results indicated that the genetically linked CTXA2B acts as a useful mucosal adjuvant, and that the FimH adhesin-CTXA2B chimaeric protein could be a potential component in future UPEC **vaccine** development.

L30 ANSWER 10 OF 71 CAPLUS COPYRIGHT 2006 ACS on STN
2003:6139 Document No. 138:68275 Mutant forms of enterotoxin (EtxB) and **cholera toxin** (**CtxB**), and their therapeutic uses as target site-specific carriers. Hirst, Timothy Raymond (University of Bristol, UK). PCT Int. Appl. WO 2003000899 A1 20030103, 84 pp.
DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2.
APPLICATION: WO 2002-GB2829 20020620. PRIORITY: GB 2001-15382 20010622.

AB The present invention describes the use of a mutant form of enterotoxin subunit B (EtxB) or **cholera toxin** subunit B (**CtxB**) to deliver an agent to a target cell wherein the mutant has GM-1 binding activity, and a reduced immunogenic and immunomodulatory activity relative to the wild type form of EtxB or **CtxB**. Specifically, the mutant **CtxB** with His to Ala substitution at position 57 is severely defective as an immunomodulator, and the holotoxin exhibits ablated toxicity, and retains the ability to bind with high

affinity to GM-1. The invention further discloses that EtxB or an EtxB(H57A) are able to act as trafficking mols. that facilitates delivery of exogenous epitopes into the endogenous pathway of class I antigen processing and presentation.

L30 ANSWER 11 OF 71 CAPLUS COPYRIGHT 2006 ACS on STN
2003:982940 Document No. 140:158418 Novel type of specialized transduction for CTX.vphi. or its satellite phage RS1 mediated by filamentous phage VGJ.vphi. in Vibrio cholerae. Campos, Javier; Martinez, Eriel; Marrero, Karen; Silva, Yussuan; Rodriguez, Boris L.; Suzarte, Edith; Ledon, Talena; Fando, Rafael (Departmento de Genetica, Centro Nacional de Investigaciones Cientificas, Havana, AP 6412, Cuba). Journal of Bacteriology, 185(24), 7231-7240 (English) 2003. CODEN: JOBAAY. ISSN: 0021-9193. Publisher: American Society for Microbiology.
AB The main virulence factor of Vibrio cholerae, the **cholera toxin**, is encoded by the ctxAB operon, which is contained in the genome of the lysogenic filamentous phage CTX.vphi.. This phage transmits ctxAB genes between V. cholerae bacterial populations that express toxin-coregulated pilus (TCP), the CTX.vphi. receptor. In investigating new forms of ctxAB transmission, we found that V. cholerae filamentous phage VGJ.vphi., which uses the mannose-sensitive hemagglutinin (MSHA) pilus as a receptor, transmits CTX.vphi. or its satellite phage RS1 by an efficient and highly specific TCP-independent mechanism. This is a novel type of specialized transduction consisting in the site-specific cointegration of VGJ.vphi. and CTX.vphi. (or RS1) replicative forms to produce a single hybrid mol., which generates a single-stranded DNA hybrid genome that is packaged into hybrid viral particles designated HybP.vphi. (for the VGJ.vphi./CTX.vphi. hybrid) and HybRS.vphi. (for the VGJ.vphi./RS1 hybrid). The hybrid phages replicate by using the VGJ.vphi. replicating functions and use the VGJ.vphi. capsid, retaining the ability to infect via MSHA. The hybrid phages infect most tested strains more efficiently than CTX.vphi., even under in vitro optimal conditions for TCP expression. Infection and lysogenization with HybP.vphi. revert the V. cholerae live attenuated **vaccine** strain 1333 to virulence. Our results reinforce that TCP is not indispensable for the acquisition of CTX.vphi.. Thus, we discuss an alternative to the current accepted evolutionary model for the emergence of new toxigenic strains of V. cholerae and the importance of our findings for the development of an environmentally safer live attenuated cholera **vaccine**.

L30 ANSWER 12 OF 71 MEDLINE on STN DUPLICATE 4
2003440542. PubMed ID: 14500467. Construction and evaluation of a safe, live, oral Vibrio cholerae **vaccine** candidate, IEM108. Liang Weili; Wang Shixia; Yu Fenggang; Zhang Lijuan; Qi Guoming; Liu Yanqing; Gao Shouyi; Kan Biao. (Priority Laboratory of Medical Molecular Bacteriology, Ministry of Health, National Institute for Communicable Disease Control and Prevention, Chinese Center for Disease Control and Prevention, Beijing 102206, People's Republic of China.) Infection and immunity, (2003 Oct) Vol. 71, No. 10, pp. 5498-504. Journal code: 0246127. ISSN: 0019-9567. Pub. country: United States. Language: English.
AB IEM101, a Vibrio cholerae O1 El Tor Ogawa strain naturally deficient in CTXPhi, was previously selected as a live cholera **vaccine** candidate. To make a better and safer **vaccine** that can induce protective immunity against both the bacteria and **cholera toxin** (CT), a new **vaccine** candidate, IEM108, was constructed by introducing a **ctxB** gene and an El Tor-derived rstR gene into IEM101. The **ctxB** gene codes for the protective antigen CTB subunit, and the rstR gene mediates phage immunity. The stable expression of the two genes was managed by a chromosome-plasmid lethal balanced system based on the housekeeping gene thyA. Immunization studies indicate that IEM108 generates good immune responses against both the bacteria and CT. After a single-dose intraintestinal vaccination with 10(9) CFU of IEM108, both anti-CTB immunoglobulin G and vibriocidal

antibodies were detected in the immunized-rabbit sera. However, only vibriocidal antibodies are detected in rabbits immunized with IEM101. In addition, IEM108 but not IEM101 conferred full protection against the challenges of four wild-type toxigenic strains of V. cholerae O1 and 4 micro g of CT protein in a rabbit model. By introducing the rstR gene, the frequency of conjugative transfer of a recombinant El Tor-derived RS2 suicidal plasmid to IEM108 was decreased 100-fold compared to that for IEM101. This indicated that the El Tor-derived rstR cloned in IEM108 was fully functional and could effectively inhibit the El Tor-derived CTXPhi from infecting IEM108. Our results demonstrate that IEM108 is an efficient and safe live oral cholera **vaccine** candidate that induces antibacterial and antitoxic immunity and CTXPhi phage immunity.

L30 ANSWER 13 OF 71 SCISEARCH COPYRIGHT (c) 2006 The Thomson Corporation on STN

2003:672586 The Genuine Article (R) Number: 705JV. Antigen-specific immunoglobulin A antibodies secreted from circulating B cells are an effective marker for recent local immune responses in patients with cholera: Comparison to antibody-secreting cell responses and other immunological markers. Qadri F (Reprint); Ryan E T; Faruque A S G; Ahmed F; Khan A I; Islam M M; Akramuzzaman S M; Sack D A; Calderwood S B. ICDDR, Div Sci Lab, GPO Box 128, Dhaka 1000, Bangladesh (Reprint); Int Ctr Diarrhoeal Dis Res, Ctr Hlth & Populat Res, Dhaka 1212, Bangladesh; Massachusetts Gen Hosp, Trop & Geog Med Ctr, Boston, MA 02114 USA; Massachusetts Gen Hosp, Div Infect Dis, Boston, MA 02114 USA; Harvard Univ, Sch Publ Hlth, Dept Immunol & Infect Dis, Boston, MA 02115 USA; Harvard Univ, Sch Med, Dept Microbiol & Mol Genet, Boston, MA 02115 USA. INFECTION AND IMMUNITY (AUG 2003) Vol. 71, No. 8, pp. 4808-4814. ISSN: 0019-9567. Publisher: AMER SOC MICROBIOLOGY, 1752 N ST NW, WASHINGTON, DC 20036-2904 USA. Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Gut-derived lymphocytes transiently migrate through the peripheral circulation before homing back to mucosal sites and can be detected using an ELISPOT-based antibody secreting cell (ASC) assay. Alternatively, transiently circulating lymphocytes may be cultured in vitro, and culture supernatants may be assayed for antigen-specific responses (antibody in lymphocyte supernatant [ALS] assay). The ALS assay has not been validated extensively in natural mucosal infection, nor has the ALS response been compared to the ASC assay and other cholera-specific immunological responses. Accordingly, we examined immune responses in 30 adult patients with acute cholera in Bangladesh, compared with 10 healthy controls, measuring ALS-immunoglobulin A (IgA), ASC-IgA, and serum and fecal IgA responses to two potent Vibrio cholerae immunogens, the nontoxic B subunit of **cholera toxin** (**CtxB**) and lipopolysaccharide (LPS) and a weaker V. cholerae immunogen, the mannose-sensitive hemagglutinin (MSHA). We found significant increases of anti-**CtxB**, anti-LPS, and anti-MSHA IgA in supernatants of lymphocytes cultured 7 days after onset of cholera using the ALS assay. We found that ALS and ASC responses correlated extremely well; both had comparable sensitivities as the vibriocidal responses, and both procedures were more sensitive than fecal IgA measurements. An advantage of the ALS assay for studying mucosal immune responses is the ability to freeze antibodies in supernatants for subsequent evaluation; like the ASC assay, the ALS assay can distinguish recent from remote mucosal infection, a distinction that may be difficult to make in endemic settings using other procedures.

L30 ANSWER 14 OF 71 MEDLINE on STN DUPLICATE 5

2003139735. PubMed ID: 12654855. Mucosal immunization with a genetically engineered pertussis toxin S1 fragment-**cholera toxin** subunit B chimeric protein. Lee Song F; Halperin Scott A; Salloum Danny F; MacMillan Ann; Morris Annette. (Department of Applied Oral Sciences, Faculty of Dentistry, Dalhousie University, Halifax, Nova Scotia, Canada

B3H 3J5.. song.lee@dal.ca) . Infection and immunity, (2003 Apr) Vol. 71, No. 4, pp. 2272-5. Journal code: 0246127. ISSN: 0019-9567. Pub. country: United States. Language: English.

AB A chimeric protein consisting of a divalent pertussis toxin (PT) S1 fragment linked to the **cholera toxin** (Ctx) A(2)B fragment was constructed. The chimera induced a mucosal immunoglobulin A (IgA) and a serum IgG immune response to PT and **CtxB** in BALB/c mice following intranasal immunization. The immune sera neutralized PT in vitro. In the mouse model of Bordetella pertussis respiratory infection, the chimera-immunized animals showed a significant reduction in bacterial lung counts (P = 0.01) from that of the sham control group. Thus, a divalent S1 fragment CtxA2B chimera is an immunogenic antigen and can elicit a protective immunity.

L30 ANSWER 15 OF 71 EMBASE DUPLICATE 6

2003367900 EMBASE Expression and characterization of uropathogenic Escherichia coli adhesin protein linked to **cholera toxin** A2B subunits in Escherichia coli TB1. Lee Y.-H.; Ryu D.-K.; Kim B.-O.; Pyo S.. S. Pyo, Division of Immunopharmacology, College of Pharmacy, SungKyunKwan University, Suwon, Kyunggi-do 440-746, Korea, Republic of. snpyo@skku.ac.kr. Journal of Microbiology and Biotechnology Vol. 13, No. 4, pp. 552-559 2003.

Refs: 32.

ISSN: 1017-7825. CODEN: JOMBES

Pub. Country: Korea, Republic of. Language: English. Summary Language: English.

Entered STN: 20030925. Last Updated on STN: 20030925

AB The FimH subunit of type 1-fimbriated Escherichia coli (E. coli) has been determined as a major cause for urinary tract infections. Thus, to produce a possible **vaccine** antigen against urinary tract infections, the fimH gene was genetically coupled to the ctxa2b gene and cloned into a pMAL-p2E expression vector. The chimeric construction of pMALfimH/ctxa2b was then transformed into E. coli K-12 TB1 and its nucleotide sequence was verified. A fusion protein, based on fusing adhesin to the **cholera toxin** subunit A2B (CTXA2B), was induced with 0.01 mM isopropyl-β-D-thiogalactoside (IPTG) for 4 h at 37°C to yield a soluble fusion protein. The fusion protein was then purified by affinity chromatography. The expressed fusion protein was confirmed by SDS-PAGE and Western blotting using antibodies to the maltose binding protein (MBP) or the **cholera toxin** subunit B (**CTXB**), plus the N-terminal amino acid sequence was also analyzed. The orderly-assembled fusion protein was confirmed by a modified G(MI)-ganglioside ELISA, using antibodies to adhesin. The results indicated that the purified fusion protein was an adhesin/CTXA2B protein containing E. coli adhesin and the G(MI)-ganglioside binding activity of **CTXB**. Accordingly, this adhesin/CTXA2B protein may be a potential antigen for oral immunization against uropathogenic E. coli.

L30 ANSWER 16 OF 71 EMBASE DUPLICATE 7

2003346967 EMBASE Construction and evaluation of the biosafe and live oral **vaccine** candidate of El Tor Vibrio cholerae IEM108. Liang W.-L.; Kan B.; Yu F.-G.; Qi G.-M.; Liu Y.-Q.; Gao S.-Y.. B. Kan, Lab. Med. Molec. Bacteriol. Min. H., Natl. Inst. Communic. Dis. Ctrl./P., Chinese Ctr. for Dis. Ctrl./Prev., Beijing 102206, China. kanb@btamail.net.cn. Chinese Journal of Microbiology and Immunology Vol. 23, No. 7, pp. 522-528 30 Jul 2003.

Refs: 15.

ISSN: 0254-5101. CODEN: ZWMZDP

Pub. Country: China. Language: Chinese. Summary Language: English; Chinese.

Entered STN: 20030918. Last Updated on STN: 20030918

AB Objective: To develop an improved attenuated oral Vibrio cholerae **vaccine** candidate which is immune to CTXϕ infection and elicits both antibacterial and antitoxic immunity. Methods: Based on the nontoxigenic and thyA gene deletion strain IEM101-T developed from IEM101, an El Tor biotype **vaccine** candidate, we constructed a chromosome-plasmid balanced lethal system by using thyA gene of E. coli as selection pressure to clone rstR gene, encoding CTXϕ phage immunity, and **ctxB** gene, encoding **cholera toxin** subunit B. In immunized rabbits, anti-CTB IgG antibody and vibriocidal antibody were detected to evaluate the immunogenicity of IEM108. A control-challenged study in rabbits was used to estimate the protection of IEM108. Results: The recombinant plasmid carrying **ctxB**, rstR and E. coli thyA' was stablely maintained in IEM101-T. CTB was detected by GM1-ELISA and expressed. Animal experiments showed that IEM108 could trigger high level of the serum anti-CTB IgG antibody and vibriocidal antibody, and offered full protection against challenges with 4 wild-type toxigenic strains of different biotypes and serogroups, and at least 4μg CT. Conclusion: By using a chromosome-plasmid balanced lethal system, a biosafe and live oral Vibrio cholerae **vaccine** candidate, IEM108 was constructed, which has induced immunity to CTXϕ infection and expresses CTB subunit stabely. Animal test showed that IEM108 was safe, immunogenic and highly protective and seemed be a well-prospective candidate eliciting both antibacterial and antitoxic immunity.

L30 ANSWER 17 OF 71 CAPLUS COPYRIGHT 2006 ACS on STN

2004:524595 Document No. 141:241687 Induction of a systemic IgG and secretory IgA responses in mice by peroral immunization with uropathogenic Escherichia coli adhesin protein coupled to **cholera toxin** A2B subunits. Lee, Yong-Hwa; Kim, Byung-Oh; Rhee, Dong-Kwon; Pyo, Suhkneung (Division of Immunopharmacology, College of Pharmacy, SungKyunKwan University, Suwon, Kyunggi-do, 440-746, S. Korea). Journal of Applied Pharmacology, 11(3), 157-162 (English) 2003. CODEN: JOAPA6. ISSN: 1225-6110. Publisher: Korean Society of Applied Pharmacology.

AB The generation of secretory IgA antibodies (Abs) for specific immune protection of mucosal surfaces depends on stimulation of the mucosal immune system, but this is not effectively achieved by parenteral or even oral administration of most soluble antigens. Thus, to produce a possible **vaccine** antigen against urinary tract infections, the uropathogenic E. coli (UPEC) adhesin was genetically coupled to the ctxa2b gene and cloned into a pMAL-p2E expression vector. The chimeric construction of pMALfimHlctxa2b was then transformed into E. coli K-12 TB1 and its nucleotide sequence was verified. The chimeric protein was then purified by applying the affinity chromatog. The purified chimeric protein was confirmed by SDS-PAGE and western blotting. The orderly-assembled chimeric protein was confirmed by a modified GMI ganglioside ELISA using antibodies to adhesin. The results indicate that the purified chimeric protein was an adhesin/CTXa2B protein containing UPEC adhesin and the GMI ganglioside binding activity of **CTXB**. The study also demonstrates that peroral administration of this chimeric immunogen in mice elicited high levels of secretory IgA and serum IgG Abs to the UPEC adhesin. Apparently, the genetically linked CTXA2B acts as a useful mucosal adjuvant, and the adhesin/CTXA2B chimeric protein might be a potential antigen for oral immunization against UPEC.

L30 ANSWER 18 OF 71 SCISEARCH COPYRIGHT (c) 2006 The Thomson Corporation on STN

2002:421293 The Genuine Article (R) Number: 551MB. A kinetic model of intermediate formation during assembly of **cholera toxin** B-subunit pentamers. Lesieur C; Cliff M J; Carter R; James R F L; Clarke A R; Hirst T R (Reprint). Univ Bristol, Sch Med Sci, Dept Pathol, Bristol

BS8 1TD, Avon, England (Reprint); Univ Leicester, Dept Surg, Leicester LE2 7LX, Leics, England; Univ Bristol, Sch Med Sci, Dept Microbiol, Bristol BS8 1TD, Avon, England; Univ Bristol, Sch Med Sci, Dept Biochem, Bristol BS8 1TD, Avon, England. JOURNAL OF BIOLOGICAL CHEMISTRY (10 MAY 2002) Vol. 277, No. 19, pp. 16697-16704. ISSN: 0021-9258. Publisher: AMER SOC BIOCHEMISTRY MOLECULAR BIOLOGY INC, 9650 ROCKVILLE PIKE, BETHESDA, MD 20814-3996 USA. Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB **Cholera toxin** is the most important virulence factor produced by Vibrio cholerae. The pentameric B-subunit of the toxin can bind to GM1-ganglioside receptors, leading to toxin entry into mammalian cells. Here, the in vitro disassembly and reassembly of **CtxB**(5) (the B subunit pentamer of **cholera toxin**) is investigated. When **CtxB**(5) was acidified at pH 1.0 and then neutralized, the B-subunits disassembled and could no longer migrate as SDS-stable pentamers on polyacrylamide gels or be captured by GM1. However, continued incubation at neutral pH resulted in the B-subunits regaining the capacity to be detected by GM1 enzyme-linked immunosorbent assay (t(1/2) similar to 8 min) and to migrate as SDS-stable pentamers (t(1/2) similar to 15 min). Time-dependent changes in Trp fluorescence intensity during B-subunit reassembly occurred with a half-time of similar to8 min, similar to that detected by GM1 enzyme-linked immunosorbent assay, suggesting that both methods monitor earlier events than B-pentamer formation alone. Based on the Trp fluorescence intensity measurements, a kinetic model of the pathway of **CtxB**(5) reassembly was generated that depended on trans to cis isomerization of Pro-93 to give an interface capable of subunit-subunit interaction. The model suggests formation of intermediates in the reaction, and these were successfully detected by glutaraldehyde cross-linking.

L30 ANSWER 19 OF 71 MEDLINE on STN DUPLICATE 8

2002294632. PubMed ID: 12034098. Comparison of mucosal and systemic humoral immune responses after transcutaneous and oral immunization strategies. John Manohar; Bridges Emily A; Miller Andy O; Calderwood Stephen B; Ryan Edward T. (Tropical & Geographic Medicine Center, Division of Infectious Diseases, Jackson 504, Massachusetts General Hospital, 55 Fruit Street, Boston, MA 02114, USA.) Vaccine, (2002 Jun 21) Vol. 20, No. 21-22, pp. 2720-6. Journal code: 8406899. ISSN: 0264-410X. Pub. country: England: United Kingdom. Language: English.

AB In order to compare the ability of transcutaneous and oral immunization strategies to induce mucosal and systemic immune responses, we inoculated mice transcutaneously with **cholera toxin** (CT) or the non-toxic B subunit of **cholera toxin** (**CtxB**), or orally with Peru2(pETR1), an attenuated **vaccine** strain of Vibrio cholerae expressing **CtxB**. In addition, we also evaluated dual immunization regimens (oral inoculation with transcutaneous boosting, and transcutaneous immunization with oral boosting) in an attempt to optimize induction of both mucosal and systemic immune responses. We found that transcutaneous immunization with purified **CtxB** or CT induces much more prominent systemic IgG anti-**CtxB** responses than does oral inoculation with a **vaccine** vector strain of V. cholerae expressing **CtxB**. In comparison, anti-**CtxB** IgA in serum, stool and bile were comparable in mice either transcutaneously or orally immunized. Overall, the most prominent systemic and mucosal anti-**CtxB** responses occurred in mice that were orally primed with Peru2(pETR1) and transcutaneously boosted with CT. Our results suggest that combination oral and transcutaneous immunization strategies may most prominently induce both mucosal and systemic humoral responses.

L30 ANSWER 20 OF 71 MEDLINE on STN DUPLICATE 9

2002676467. PubMed ID: 12437076. Expression of **cholera toxin** B subunit in transgenic tomato plants. Jani Dewal; Meena

Laxman Singh; Rizwan-ul-Haq Quazi Mohammad; Singh Yogendra; Sharma Arun K; Tyagi Akhilesh K. (Department of Plant Molecular Biology, University of Delhi, New Delhi, India.) Transgenic research, (2002 Oct) Vol. 11, No. 5, pp. 447-54. Journal code: 9209120. ISSN: 0962-8819. Pub. country: Netherlands. Language: English.

AB **Cholera toxin**, secreted by Vibrio cholerae, consists of A and B subunits. The latter binds to G(M1)-ganglioside receptors as a pentamer (approximately 55 kDa). Tomato plants were transformed with the gene encoding **cholera toxin** B subunit (**ctxB**) along with an endoplasmic reticulum retention signal (SEKDEL) under the control of the CaMV 35S promoter via Agrobacterium-mediated transformation. PCR and Southern analysis confirmed the presence of the **ctxB** gene in transformed tomato plants. Northern analysis showed the presence of the **ctxB**-specific transcript. Immunoblot assays of the plant-derived protein extract showed the presence of **cholera toxin** subunit B (CTB) with mobility similar to purified CTB from V. cholerae. Both tomato leaves and fruits expressed CTB at levels up to 0.02 and 0.04% of total soluble protein, respectively. The G(M1)-ELISA showed that the plant-derived CTB bound specifically to G(M1)-ganglioside receptor, suggesting that it retained its native pentameric form. This study forms a basis for exploring the utility of CTB to develop tomato-based edible **vaccines** against cholera.

L30 ANSWER 21 OF 71 CAPLUS COPYRIGHT 2006 ACS on STN

2003:84556 Document No. 138:336065 Preparation and immunogenicity study of HspA-**CtxB** fusion protein to Helicobacter pylori in mice. Li, Mingfeng; Liu, Xinmei; Ling, Zhen; He, Zhiyong; Shen, Xudong; Sun, Jianxin; Wu, Xiangfu (Department of Gastroenterology, 455 Hospital of PLA, Shanghai, 200052, Peop. Rep. China). Zhonghua Weishengwuxue He Mianyixue Zazhi, 22(4), 398-402 (Chinese) 2002. CODEN: ZWMZDP. ISSN: 0254-5101. Publisher: Weishenbu Beijing Shengwu Zhipin Yanjiuso.

AB The immune response in mice fed orally with the conjugated antigen of HspA-**CtXB** (HCT) as well as the absorption of the antigen in the small intestine of mice was studied. LlkkA recombinant strain which could express bivalent antigen of HspA and **CtxB** subunit was constructed. HspA and hct gene was amplified by PCR. The DNA products of HspA and **CtxB** were inserted into a prokaryotic expression vector pET-22b(+) resp., and then transfected into E.coli strain BL-21 (DE3) to express HCT fusion protein. Hct gene was sequenced as 726 base pairs, the fusion protein encoded polypeptides of 242 amino acid residues, corresponding to calculated mol. masses(Mr) of 30 x 103. Western blot anal. of the recombinant protein HCT confirmed that it could he specifically recognized by the serum of H.pylori-infected patients. HspA and HCT labeled 125I were orally delivered into the stomach of mice resp., and the radioactivity of 125I in serum of each mouse was detected in different periods: 5 min, 10 min, 15 min, 30 min, 60 min and 90 min. Antibody response in animals immunized with recombinant HCT or HspA was determined by ELISA. In the group of HCT, serum IgA and IgG were 2.98±0.35 and 4.38±0.56 resp.; fecal IgA was 2.89±0.68. In the HspA, serum IgA and IgG were 0.38±0.15 and 0.45±0.16 resp.; fecal IgA was 0.69±0.23. In normal control group, serum IgA and IgG were 0.06±0.02 and 0.09±0.06 resp.; fecal IgA 0.12±0.03. These results indicate that HCT can be immediately absorbed in a large amount from small intestine of mice, and high value of HspA-specific antibodies are produced by orally immunization with HCT. The recombinant fusion protein HCT can be used as an effective oral **vaccine** for prevention and treatment of infection of H.pylori.

L30 ANSWER 22 OF 71 EMBASE COPYRIGHT (c) 2006 Elsevier B.V. All rights reserved on STN DUPLICATE 10

2002410564 EMBASE In vitro expression of the **ctxB** toxin gene towards the development of a DNA **vaccine** against cholera. Syahril N.A.; Mariana N.S.; Sidhu H.S.; Rosli R.. R. Rosli, Faculty of

Medicine/Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia. rozita@medic.upm.edu.my. Molecular Biology Today Vol. 3, No. 3, pp. 71-77 2002.
Refs: 23.
ISSN: 1468-5698. CODEN: MBTOC8
Pub. Country: United Kingdom. Language: English. Summary Language: English.
Entered STN: 20021202. Last Updated on STN: 20021202

AB The complete eradication of cholera is an unachievable goal because it is now firmly established that there are environmental reservoirs for Vibrio cholerae. Although there are effective treatments for this disease, they are expensive and impractical in times of epidemic. All these points lead to the fact that the development of a safe, cheap, and efficient **vaccine** is probably the best solution to the problem. A new generation of **vaccine**, termed DNA **vaccine**, would probably be a better alternative to the traditional **vaccines**. In this study, the focus is on the **ctxB**, the gene encoding the B subunit **cholera toxin** as a potential candidate for the DNA **vaccine** against cholera. The **ctxB** gene is required for the binding of the **Cholera Toxin** (CT) to the eukaryotic cell and facilitates the entry of the active toxin (CTXA) into the host cell which causes the profuse diarrheal symptom. The **ctxB** gene was cloned in pVax 1 (Invitrogen), and proven to be in the correct orientation. Subsequently, expression of the B subunit toxin in vitro, was successfully carried out using 10 and 20 ml of Effectene® (Qiagen) reagent with 0.4 mg pVax/**ctxB**, 90 hours post transfection in COS-7 cells. The results showed that the pentamer size of the **ctxB** (58 kDa) was expressed instead of its single monomer of 11.6 kDa. This means that the mechanism of the eukaryotic expression system in vitro was able to produce this end-product by successfully processing the binding of five single peptides of the **ctxB**. Further investigations involving this potential DNA **vaccine** against cholera is currently underway, including the production of antibody in animal models.

L30 ANSWER 23 OF 71 MEDLINE on STN DUPLICATE 11
2001248162. PubMed ID: 11292779. Escherichia coli heat-labile enterotoxin B subunit is a more potent mucosal adjuvant than its vlosely related homologue, the B subunit of **cholera toxin**. Millar D G; Hirst T R; Snider D P. (Department of Pathology and Molecular Medicine, McMaster University, Hamilton, Ontario, Canada L8N 3Z5.. dmillar@uhnres.utoronto.ca) . Infection and immunity, (2001 May) Vol. 69, No. 5, pp. 3476-82. Journal code: 0246127. ISSN: 0019-9567. Pub. country: United States. Language: English.

AB Although **cholera toxin** (Ctx) and Escherichia coli heat-labile enterotoxin (Etx) are known to be potent mucosal adjuvants, it remains controversial whether the adjuvanticity of the holotoxins extends to their nontoxic, receptor-binding B subunits. Here, we have systematically evaluated the comparative adjuvant properties of highly purified recombinant EtxB and **CtxB**. EtxB was found to be a more potent adjuvant than **CtxB**, stimulating responses to hen egg lysozyme when the two were coadministered to mice intranasally, as assessed by enhanced serum and secretory antibody titers as well as by stimulation of lymphocyte proliferation in spleen and draining lymph nodes. These results indicate that, although structurally very similar, EtxB and **CtxB** have strikingly different immunostimulatory properties and should not be considered equivalent as prospective **vaccine** adjuvants.

L30 ANSWER 24 OF 71 MEDLINE on STN DUPLICATE 12
2001192726. PubMed ID: 11160664. Protective mucosal immunity to ocular herpes simplex virus type 1 infection in mice by using Escherichia coli heat-labile enterotoxin B subunit as an adjuvant. Richards C M; Aman A T;

Hirst T R; Hill T J; Williams N A. (Department of Pathology and Microbiology, School of Medical Sciences, University of Bristol, Bristol BS8 1TD, United Kingdom.. Claire.M.Richards@bristol.ac.uk) . Journal of virology, (2001 Feb) Vol. 75, No. 4, pp. 1664-71. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB The potential of nontoxic recombinant B subunits of **cholera toxin** (rCtxB) and its close relative Escherichia coli heat-labile enterotoxin (rEtxB) to act as mucosal adjuvants for intranasal immunization with herpes simplex virus type 1 (HSV-1) glycoproteins was assessed. Doses of 10 microg of rEtxB or above with 10 microg of HSV-1 glycoproteins elicited high serum and mucosal anti-HSV-1 titers comparable with that obtained using **CtxB** (10 microg) with a trace (0.5 microg) of whole toxin (Ctx-**CtxB**). By contrast, doses of rCtxB up to 100 microg elicited only meager anti-HSV-1 responses. As for Ctx-**CtxB**, rEtxB resulted in a Th2-biased immune response with high immunoglobulin G1 (IgG1)/IgG2a antibody ratios and production of interleukin 4 (IL-4) and IL-10 as well as gamma interferon by proliferating T cells. The protective efficacy of the immune response induced using rEtxB as an adjuvant was assessed following ocular challenge of immunized and mock-immunized mice. Epithelial disease was observed in both groups, but the immunized mice recovered by day 6 whereas mock-immunized mice developed more severe corneal disease leading to stromal keratitis. In addition, a significant reduction in the incidence of lid disease and zosteriform spread was observed in immunized animals and there was no encephalitis compared with 95% encephalitis in mock-immunized mice. The potential of such mucosal adjuvants for use in human **vaccines** against pathogens such as HSV-1 is discussed.

L30 ANSWER 25 OF 71 MEDLINE on STN DUPLICATE 13

2001446632. PubMed ID: 11494170. Local production of anti-vibrio cholerae mucosal antibody in reproductive tract tissues after cholera. Ryan E T; Bridges E A; Crean T I; Gausia K; Hamadani J D; Aziz A; Hawkes S; Begum M; Bogaerts J; Faruque S M; Salam M A; Fuchs G J; Calderwood S B. (Tropical and Geographic Medicine Center, Division of Infectious Diseases, Massachusetts General Hospital, Boston, MA 02114, USA.. etryan@partners.org) . The Journal of infectious diseases, (2001 Sep 1) Vol. 184, No. 5, pp. 643-7. Electronic Publication: 2001-08-09. Journal code: 0413675. ISSN: 0022-1899. Pub. country: United States. Language: English.

AB To investigate whether intestinal presentation of an antigen by Vibrio cholerae, a noninvasive organism, could induce an anatomically distant mucosal immune response in reproductive tract tissues, the endocervical immune responses of women in Bangladesh were evaluated after cholera. Endocervical secretions were analyzed for secretory IgA (sIgA) antibody against the B subunit of **cholera toxin** (**CtxB**) in 9 women with cholera and 8 women with diarrhea caused by neither V. cholerae nor heat labile enterotoxin-producing Escherichia coli. Women infected with V. cholerae developed significant sIgA anti-**CtxB** responses in endocervical samples (P< or =.02). Antibody subtype analysis of endocervical IgA was consistent with local mucosal production (P< or =.001). Women with cholera did not develop sIgA anti-**CtxB** responses in serum. The ability to generate specific mucosal immune responses in reproductive tract tissues after intestinal presentation of antigen could facilitate development of **vaccines** effective against reproductive tract pathogens.

L30 ANSWER 26 OF 71 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN DUPLICATE 14

2001:290633 Document No.: PREV200100290633. A candidate oral **vaccine** to Helicobacter pylori: Fusion protein of HspA and **CtxB**. Li Ming-Feng; He Zhi-Yong; Ling Zheng; Wang Jiao-Yang; Sheng Xu-Dong; Yang Guan-Zhen; Wu Xiang-Fu [Reprint author]. Shanghai Institute of Biochemistry, Chinese Academy of Sciences, Shanghai, 200031, China.

xfwu@sunm.shcnc.ac.cn. Shengwu Huaxue yu Shengwu Wuli Xuebao, (May, 2001) Vol. 33, No. 3, pp. 360-364. print.
ISSN: 0582-9879. Language: Chinese.

AB Heat-shock protein A subunit (HspA), an effective immunogen may stimulate the immunoresponse in human body against challenge of H. pylori. The B subunit of **cholera toxin** (**CtxB**) has been proved to be a potent mucosal immunogen, act as an adjuvant for **vaccine** targeted for delivery to the mucosal-associated lymphoid tissue. A recombinant plasmid expressing bivalent antigen of HspA and **CtxB** subunit was constructed as follows. hspA and **ctxB** gene was amplified by PCR. The DNA products of hspA and **ctxB** were inserted in the prokaryotic expression vector pET-22b(+), respectively, and then the resulted recombinant plasmid expressing a fusion protein named HCT was transformed into the E. coli strain BL-21(DE3). hct gene was measured to be 708 base pairs long, and the fusion protein encoding a polypeptide of 236 amino acid residues, corresponded to a calculated molecular masses of 30 kD. Western blot analysis of the recombinant protein HCT confirmed that it could be specifically recognized by the serum of H. pylori-infected patients. HspA and HCT labelled 125I were orally administered into the stomach of mice, respectively, and the radioactivity of 125I in serum of each mouse was assayed at intervals: 15 min, 30 min, 60 min, 90 min and 120 min. The result indicated that there were high radioactivity counts in the groups of HCT than that of HspA ($P < 0.001$). This result suggests that the **CtxB** may enhance the volume of HspA absorbed from the intestine of mice, therefore the recombinant fusion protein HCT may be an effective oral **vaccine** for prevention and treatment against the infection of H. pylori.

L30 ANSWER 27 OF 71 MEDLINE on STN DUPLICATE 15

2001206192. PubMed ID: 11222115. Peroral immunization with Helicobacter pylori adhesin protein genetically linked to **cholera toxin** A2B subunits. Kim B O; Shin S S; Yoo Y H; Pyo S. (School of Pharmacy, Sung Kyun Kwan University, Suwon, 440-746, Kyunggi-Do, South Korea.) Clinical science (London, England : 1979), (2001 Mar) Vol. 100, No. 3, pp. 291-8. Journal code: 7905731. ISSN: 0143-5221. Pub. country: England: United Kingdom. Language: English.

AB Helicobacter pylori is a major cause of gastric-associated diseases. To evaluate the efficacy of a possible **vaccine** antigen against H. pylori infection, the chimaeric construct adhesin--CTXA2B, derived from H. pylori adhesin genetically coupled to **cholera toxin** (CTX) subunits A2 and B (CTXA2B), was expressed in Escherichia coli as an insoluble recombinant chimaeric protein. The protein was then purified by denaturation, renaturation and size-exclusion chromatography. The composition of purified adhesin--CTXA2B was verified by SDS/PAGE and Western blotting with antibodies to antigenic components of adhesin and **CTXB**, and confirmed as a chimaeric protein with G(M1)-ganglioside binding activity and adhesin epitopes by a G(M1)-ELISA developed using antibodies to adhesin. Oral immunization of mice with adhesin--CTXA2B induced higher levels of mucosal IgA and serum IgG antibodies to H. pylori adhesin and to **CTXB** than in mice immunized with adhesin or CTXA2B alone. Adhesin--CTXA2B was also demonstrated to be a potential protective antigen in a mouse model of H. pylori infection. The immunization of mice with adhesin--CTXA2B protected 62.5% of mice infected with H. pylori SS1 strain, whereas adhesin immunization was not able to confer protection to mice. This protection may be correlated with high levels of mucosal IgA and serum IgG antibodies against H. pylori adhesin. Taken together, the results indicate that the genetically linked CTXA2B acts as a useful mucosal adjuvant, and that the adhesin-CTXA2B chimaeric protein could be a potential component in future H. pylori **vaccine** development.

L30 ANSWER 28 OF 71 MEDLINE on STN DUPLICATE 16

2000143721. PubMed ID: 10678922. In vitro and in vivo analyses of constitutive and in vivo-induced promoters in attenuated **vaccine** and vector strains of Vibrio cholerae. John M; Crean T I; Calderwood S B; Ryan E T. (Division of Infectious Diseases, Massachusetts General Hospital, Boston, Massachusetts 02114, USA.) Infection and immunity, (2000 Mar) Vol. 68, No. 3, pp. 1171-5. Journal code: 0246127. ISSN: 0019-9567. Pub. country: United States. Language: English.

AB The optimal promoter for in vivo expression of heterologous antigens by live, attenuated **vaccine** vector strains of Vibrio cholerae is unclear; in vitro analyses of promoter activity may not accurately predict expression of antigens in vivo. We therefore introduced plasmids expressing the B subunit of **cholera toxin** (**CtxB**) under the control of a number of promoters into V. cholerae **vaccine** strain Peru2. We evaluated the tac promoter, which is constitutively expressed in V. cholerae, as well as the in vivo-induced V. cholerae heat shock htpG promoter and the in vivo-induced V. cholerae iron-regulated irgA promoter. The functionality of all promoters was confirmed in vitro. In vitro antigenic expression was highest in **vaccine** strains expressing **CtxB** under the control of the tac promoter (2 to 5 microgram/ml/unit of optical density at 600 nm [OD(600)]) and, under low-iron conditions, in strains containing the irgA promoter (5 microgram/ml/OD(600)). We orally inoculated mice with the various **vaccine** strains and used anti-**CtxB** immune responses as a marker for in vivo expression of **CtxB**. The **vaccine** strain expressing **CtxB** under the control of the tac promoter elicited the most prominent specific anti-**CtxB** responses in vivo (serum immunoglobulin G [IgG], P </= 0.05; serum IgA, P </= 0.05; stool IgA, P </= 0.05; bile IgA, P </= 0.05), despite the finding that the tac and irgA promoters expressed equivalent amounts of **CtxB** in vitro. Vibriocidal antibody titers were equivalent in all groups of animals. Our results indicate that in vitro assessment of antigen expression by **vaccine** and vector strains of V. cholerae may correlate poorly with immune responses in vivo and that of the promoters examined, the tac promoter may be best suited for expression from plasmids of at least certain heterologous antigens in such strains.

L30 ANSWER 29 OF 71 EMBASE COPYRIGHT (c) 2006 Elsevier B.V. All rights reserved on STN

2000114046 EMBASE Expression and characterization of Helicobacter pylori adhesin protein linked to **cholera toxin** A2/B subunits in Escherichia coli. Kim B.O.; Sung Seup Shin; Young Hyo Yoo; Pyo S.. S. Pyo, School of Pharmacy, Sung Kyun Kwan University, Suwon 440-746, Korea, Republic of. snpyo@yurim.skku.ac.kr. Journal of Microbiology and Biotechnology Vol. 10, No. 1, pp. 56-62 2000.
Refs: 28.
ISSN: 1017-7825. CODEN: JOMBES
Pub. Country: Korea, Republic of. Language: English. Summary Language: English.
Entered STN: 20000413. Last Updated on STN: 20000413

AB The hpa gene genetically linked to the ctxa2b gene was cloned into the pTED expression vector, and the constructed pTEDhpa/ctxa2b was transformed into Escherichia coli. The fusion protein, the adhesin fused to the **cholera toxin** subunit A2B (CTXA2B) subunit, was expressed to high levels as inclusion bodies in E. coli. The expressed protein was partially purified by washing the inclusion bodies with working solution containing 8 M Urea and 0.1 M DTT. Refolding of denatured fusion protein was carried out in the presence of glutathione redox buffer. The refolded fusion protein was purified by size exclusion chromatography. The expressed fusion protein was verified by SDS- PAGE, western blotting with antibodies to both antigenic components of adhesin and **cholera toxin** subunit B (**CTXB**), and its N-terminal amino acid sequence was analyzed. The orderly assembled fusion protein was confirmed by modified G(MI)-ganglioside ELISA with Abs to

adhesin. The results indicate that the purified fusion protein is an Adhesin/CTXA2B protein containing the H. pylori adhesin and G(MI)-ganglioside binding activity of **CTXB** and the expressed fusion protein in E. coli could be easily purified by the refolding process. Its molecular weight was 168 kDa as estimated by size exclusion chromatography. The Adhesin/CTXA2B protein may be used as a candidate antigen for oral immunization against H. pylori.

L30 ANSWER 30 OF 71 CAPLUS COPYRIGHT 2006 ACS on STN
2002:420039 Document No. 137:230880 Efforts towards the development of oral cholera **vaccines** in India against the backdrop of global endeavours. Ghosh, Amit; Thungapathra, M.; Sharma, C.; Gupta, N.; Ghosh, R. K.; Mukhopadhyay, A.; Kole, H.; Nair, G. B. (Institute of Microbial Technology, Chandigarh, 160 036, India). Diarrhoeal Diseases: Research Perspectives, [Lectures delivered at the Symposium on "New Perspectives of Research in Cholera and Diarrhoeal Diseases"], New Delhi, India, Mar. 18, 1998, Meeting Date 1998, 1-16. Editor(s): Rao, N. Appaji; Ganguly, N. K. Indian National Science Academy: New Delhi, India. ISBN: 81-7319-343-6 (English) 2000. CODEN: 69CQUD.

AB A review. According to the World Health Organization more than 70 million people died of infectious diseases in 1996. One of the infectious diseases which continues to cause global concern is cholera. Though it can be controlled by improved sanitation, this goal is not attainable in most countries of the world. The development of an effective **vaccine** therefore, still remains the best solution As parenteral **vaccines** developed during the last 100 yr were found to be ineffective, in recent years two different approaches have been pursued for developing oral **vaccines**. While the first approach is based on the observation that a cholera patient develops both anti-toxic and anti-bacterial immunity, the rationale for the second approach is that convalescents develop lasting immunity against a fresh attack. Using the first approach, a combination **vaccine** comprising killed whole cells and the B subunit of **cholera toxin**, was developed. The outcome of the second approach was the development of ctxA-B+ strains of Vibrio cholerae (strains unable to synthesize the catalytic A subunit of the **cholera toxin**), which could mimic infection derived immunity in the host, when administered orally. However, all such strains were found to be reactogenic. A critical anal. of the available data indicated to us that the starting strain dets. the reactogenicity of the final construct. Hence a strain with requisite properties -completely non-reactogenic, devoid of all toxin genes and a good colonizer, was obtained after screening hundreds of isolates. The **ctxB** gene with its own up and downstream regulatory sequences, was then introduced into the chromosome of this strain at a specific locus. The recombinant strain designated VA1.3, was found to be completely non-reactogenic and 100% protective in animal studies. It was also found to be completely safe in toxicity studies conducted at the Post-Graduate Institute of Medical Education and Research, Chandigarh, under the guidance of Prof. N.K. Ganguly. The **vaccine** is now undergoing phase I trial. Approaches somewhat similar to the aforementioned ones have also been pursued by other groups in India. Thus while, Dr. B.S. Srivastava and his group (Central Drug Research Institute, Lucknow) have developed a potential subunit **vaccine** which looks promising, Dr. J. Das and coworkers (Indian Institute of Chemical Biol., Calcutta) have developed a plasmid based recombinant oral **vaccine** which has produced good results in animal studies.

L30 ANSWER 31 OF 71 CAPLUS COPYRIGHT 2006 ACS on STN
1999:736498 Document No. 131:335799 Immunomodulatory activity of B subunits of **cholera toxin**, verotoxin, and heat-labile enterotoxin. Hirst, Timothy Raymond; Williams, Neil Andrew (University of Bristol, UK). PCT Int. Appl. WO 9958145 A2 19991118, 63 pp. DESIGNATED STATES: W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU,

CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1999-GB1461 19990510. PRIORITY: GB 1998-9958 19980508; GB 1998-11954 19980603; GB 1998-12316 19980608.

AB The authors disclose the use of: (i) heat-labile enterotoxin B subunit (EtxB), **cholera toxin** B subunit (**CtxB**) or verotoxin B subunit (VtxB) in **vaccine** prepns. to alter the immune response to pathogens. In one example, the secretory IgA response to herpes virus glycoproteins is enhanced by the adjuvant activity of EtxB. In addition, the authors disclose the use of agents other than EtxB or **CtxB**, which have ganglioside GM1-binding activity, or an agent other than VtxB which has globotriosylceramide (Gb3)-binding activity for affecting intracellular signaling events.

L30 ANSWER 32 OF 71 CAPLUS COPYRIGHT 2006 ACS on STN

1999:193820 Document No. 130:220304 Avirulent strains of Vibrio cholerae 01 and non-01 serogroups for use in live **vaccines** and their construction. Kaper, James B.; Levine, Myron M. (The University of Maryland System, USA). U.S. US 5882653 A 19990316, 63 pp., Cont.-in-part of U.S. Ser. No. 133,438, abandoned. (English). CODEN: USXXAM. APPLICATION: US 1996-624601 19960729. PRIORITY: US 1983-472276 19830304; US 1984-581406 19840217; US 1986-867633 19860527; US 1989-363383 19890605; US 1990-533315 19900605; US 1992-821872 19920116; US 1992-931943 19920812; US 1993-133438 19931008; US 1993-133439 19931008; WO 1994-US11424 19941007.

AB Avirulent Vibrio cholerae strains of O1 (CVD111) and non-O1 (CVD112 and CVD112RM) serogroups suitable for **vaccine** use are described. These strains are non-toxigenic as a result of having the DNA for the **cholera toxin** core and the RS1 sequences of the **cholera toxin** locus deleted, and further having a mercury resistance marker and a sequence encoding an antigenic fragment of the toxin B subunit re-inserted in the chromosome. Further, these strains may also have the zona occludens toxin and accessory cholera enterotoxin genes deleted and retain the ability to colonize the small intestine. Methods of making the avirulent V. cholerae O1 and non-O1 strains of the invention, and cholera **vaccines** using these strains. Although remaining antigenic, these strains do not appear to be reactogenic.

L30 ANSWER 33 OF 71 CAPLUS COPYRIGHT 2006 ACS on STN

1999:457943 Document No. 131:78415 Nontoxinogenic cholera organisms and **vaccines**. Muthukumarappa, Thungapathra; Ghosh, Amit; Sharma, Charu; Gupta, Naveen; Ghosh, Ranajit K.; Mukhopadhyay, A.; Kole, Hemanta; Nair, G. B. (Council of Scientific and Industrial Research, India; National Institute of Cholera and Enteric Diseases; Department of Biotechnology, Ministry of Science and Technology, Government of India). Eur. Pat. Appl. EP 928831 A1 19990714, 18 pp. DESIGNATED STATES: R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO. (English). CODEN: EPXXDW. APPLICATION: EP 1997-309957 19971210.

AB A process for the isolation of nontoxinogenic V. cholerae strain and a process for preparing a cholera **vaccine** from said V. cholerae strain is provided. V. cholerae is isolated from the stool of a patient suffering from cholera by spreading the stool on a selector medium specific for V. cholerae.1. The non-toxinogenic V. cholerae strain is separated from the population of the V. cholerae strains isolated in the first step. The strain is deposited at Microbial Type Culture Collection (MTCC) at the Institute of Microbial Technol. (IMT), Chandigarh, India, a constituent laboratory of the applicants and has the Accession number MTCC B0010

and is also deposited at American Type Culture Collection, Rockville, Maryland, USA with the Accession number ATCC 202010. Immunogenic **cholera toxin** (ctx) B subunit gene is incorporated into the chromosomal gene hlyA (encoding hemolysin A) by genetic recombination of the strain having the Accession number MTCC B0010 (ATCC 202010) to produce the **vaccine**.

L30 ANSWER 34 OF 71 CAPLUS COPYRIGHT 2006 ACS on STN
1999:768680 Document No. 132:62844 Randomized, double-blind, placebo-controlled, multicentered trial of the efficacy of a single dose of live oral cholera **vaccine** CVD 103-HgR in preventing cholera following challenge with Vibrio cholerae O1 El Tor inaba three months after vaccination. Tacket, Carol O.; Cohen, Mitchell B.; Wasserman, Steven S.; Losonsky, Genevieve; Livio, Sofie; Kotloff, Karen; Edelman, Robert; Kaper, James B.; Cryz, Stanley J.; Giannella, Ralph A.; Schiff, Gilbert; Levine, Myron M. (Center for Vaccine Development, University of Maryland School of Medicine, Baltimore, MD, 21201, USA). Infection and Immunity, 67(12), 6341-6345 (English) 1999. CODEN: INFIBR. ISSN: 0019-9567. Publisher: American Society for Microbiology.
AB CVD 103-HgR is a live oral cholera **vaccine** strain constructed by deleting 94% of the gene for the enzymically active A subunit of **cholera toxin** from classical Inaba Vibrio cholerae O1 569B; the strain also contains a mercury resistance gene as an identifying marker. This **vaccine** was well tolerated and immunogenic in double-blind, controlled studies and was protective in open-label studies of volunteers challenged with V. cholerae O1. A randomized, double-blind, placebo-controlled, multicenter study of **vaccine** efficacy was designed to test longer-term protection of CVD 103-HgR against moderate and severe El Tor cholera in U.S. volunteers. A total of 85 volunteers (50 at the University of Maryland and 35 at Children's Hospital Medical Center/University of Cincinnati) were recruited for vaccination and challenge with wild-type V. cholerae El Tor Inaba. Volunteers were randomized in a double-blind manner to receive, with buffer, a single oral dose of either CVD 103-HgR (2+108 to 8+108 CFU) or placebo (killed E. coli K-12). About 3 mo after immunization, 51 of these volunteers were orally challenged with 105 CFU of virulent V. cholerae O1 El Tor Inaba strain N16961, prepared from a standardized frozen inoculum. Ninety-one percent of the vaccinees had a ≥4-fold rise in serum vibriocidal antibodies after vaccination. After challenge, 9 (39%) of the 23 placebo recipients and 1 (4%) of the 28 vaccinees had moderate or severe diarrhea (≥3-L diarrheal stool) (protective efficacy, 91%). A total of 21 (91%) of 23 placebo recipients and 5 (18%) of 28 vaccinees had any diarrhea (protective efficacy, 80%). Peak stool V. cholerae excretion among placebo recipients was 1.1+107 CFU/g and among vaccinees was 4.9+102 CFU/g. This **vaccine** could therefore be a safe and effective tool to prevent cholera in travelers.

L30 ANSWER 35 OF 71 MEDLINE on STN DUPLICATE 17
1999351710. PubMed ID: 10424424. Construction of a recombinant live oral **vaccine** from a non-toxigenic strain of Vibrio cholerae O1 serotype inaba biotype E1 Tor and assessment of its reactogenicity and immunogenicity in the rabbit model. Thungapathra M; Sharma C; Gupta N; Ghosh R K; Mukhopadhyay A; Koley H; Nair G B; Ghosh A. (Institute of Microbial Technology, Sector 39A, Chandigarh, India.) Immunology letters, (1999 Jun 1) Vol. 68, No. 2-3, pp. 219-27. Journal code: 7910006. ISSN: 0165-2478. Pub. country: Netherlands. Language: English.
AB The disease cholera is an important cause of mortality in many developing countries. Though it can be controlled through improved sanitation, this goal is not easily attainable in many countries. Development of an efficacious **vaccine** offers the best immediate solution. A new oral candidate **vaccine** has been constructed from a non-toxigenic strain of Vibrio cholerae El Tor, Inaba, which is not only devoid of the **cholera toxin** (CT) virulence cassette but also is

completely non-reactogenic in rabbit ileal loop assay. The strain, however, had toxR and tcpA genes. Through a series of manipulations, the **ctxB** gene of V. cholerae, responsible for the production of the 'B' subunit of the **cholera toxin** (CTB) was introduced into the cryptic hemolysin locus of the strain. The resulting strain, named **vaccine** attempt 1.3 (VA1.3), was found to be able to produce copious amounts of CTB. In the RITARD model this strain was found to be non-reactogenic and provided full protection against the challenge doses of both V. cholerae O1, classical and E1 Tor. In the immunized rabbit it invoked significant levels of anti-bacterial and anti-toxin immunity.

L30 ANSWER 36 OF 71 CAPLUS COPYRIGHT 2006 ACS on STN
1999:373884 Document No. 131:154427 The regulation of **cholera toxin** gene expression. Duan, Guangcai; Rappuoli, Rino; Gao, Shouyi; Liu, Yanqing; Qi, Guoming; Fontana, MariaRita (Department of Epidemiology, Henan Medical University, Zhengzhou, 450052, Peop. Rep. China). Henan Yike Daxue Xuebao, 33(5), 62-65 (Chinese) 1998. CODEN: HEYDE2. ISSN: 1000-1069. Publisher: Henan Yike Daxue Xuebao Bianjibu.
AB **Cholera toxin** (CT) consists of one A subunit (CT-A) and five B subunits (CT-B). The genes encoding A subunit and B subunit are named ctxA and **ctxB**. Conventional studies indicated that the transcription of ctxA and **ctxB** is one open reading frame (ORF) in the control of the promoter Pctx. In this study, we constructed several high CT expressing vectors in order to develop the new cholera **vaccines**. The **vaccine** candidate strain IEM101 (Vibrio cholerae O1, CT neg.) was transformed with these plasmids by electro-transformation, and the expression levels of CT-A and CT-B were detected with western blot. CT-B could be expressed together with CT-A under the control of different promoter systems; meanwhile, CT-B could also be expressed well even when CT-A was not expressed. Apparently, there is a promoter-like system at the upper stream of **ctxB**, and CT-B can be expressed independently. The effects of different promoters on CT expression in Vibrio cholerae are also studied. The promoters Ptac, PlacUV5, PT7 could work well in IEM101.

L30 ANSWER 37 OF 71 MEDLINE on STN DUPLICATE 18
1998062149. PubMed ID: 9400977. Salmonella typhimurium aroA recombinants and immune-stimulating complexes as **vaccine** candidates for feline immunodeficiency virus. Tijhaar E J; Huisman W; Huisman R C; Siebelink K H; Karlas J A; de Ronde A; van Herwijnen R; Mooi F R; Osterhaus A D. (National Institute of Public Health and the Environment, Bilthoven, The Netherlands.) The Journal of general virology, (1997 Dec) Vol. 78 (Pt 12), pp. 3265-75. Journal code: 0077340. ISSN: 0022-1317. Pub. country: ENGLAND: United Kingdom. Language: English.
AB Two experimental feline immunodeficiency virus (FIV) **vaccines** were tested, either alone or in combination, in four groups of cats (A-D). One **vaccine** (SL3261-FIV) was composed of live attenuated Salmonella typhimurium aroA (SL3261) strains expressing the capsid (Gag) and part of the envelope (Env) proteins of FIV. The other was composed of FIV Gag and Env proteins incorporated into immune-stimulating complexes (iscom-FIV). Cats of group A were immunized four times with SL3261-FIV. Cats of group B were immunized twice with SL3261-FIV and then twice with iscom-FIV. Cats of group C were immunized twice with SL3261 expressing the B subunit of **cholera toxin** (SL3261-**CtxB**) and then twice with iscom-FIV. Cats of group D, which served as negative controls, were immunized twice with SL3261-**CtxB** and then twice with iscom into which the Gag and Env proteins of simian immunodeficiency virus (SIV) had been incorporated (iscom-SIV). Two weeks after the last immunization, all cats were challenged with FIV. At this time, cats immunized with iscom-FIV (groups B and C) showed strong plasma antibody responses to Gag and Env, whilst these responses were weak or undetectable in the cats immunized four times with SL3261-FIV (group A). Seven weeks

after FIV challenge, Env-specific antibody responses had increased considerably in cats of all groups except group A. The mean virus loads in the cats of this group proved to be lower than those of the other groups at all time points, indicating partial protection.

L30 ANSWER 38 OF 71 MEDLINE on STN DUPLICATE 19
97321753. PubMed ID: 9178455. Induction of feline immunodeficiency virus specific antibodies in cats with an attenuated Salmonella strain expressing the Gag protein. Tijhaar E J; Siebelink K H; Karlas J A; Burger M C; Mooi F R; Osterhaus A D. (School of Biological and Medical Sciences, University of St Andrews, Scotland.) Vaccine, (1997 Apr-May) Vol. 15, No. 6-7, pp. 587-96. Journal code: 8406899. ISSN: 0264-410X. Pub. country: ENGLAND: United Kingdom. Language: English.

AB Salmonella typhimurium aroA strains (SL3261), expressing high levels of the Gag protein of feline immunodeficiency virus (FIV) fused with maltose binding protein (SL3261-MFG), were constructed using an invertible promoter system that allows the stable expression of heterologous antigens at levels toxic for bacteria. A SL3261 strain expressing the B subunit of **cholera toxin** by a similar system (SL3261-**CtxB**) served as a control in FIV-immunization experiments. Cats immunized once orally or intraperitoneally with SL3261-MFG or SL3261-**CtxB** all developed serum antibodies to SL3261 lipopolysaccharide and against maltose binding protein or the B subunit of **cholera toxin**, respectively. Two intraperitoneal immunizations with SL3261-MFG also resulted in the development of Gag specific serum antibodies. Two oral immunizations with SL3261-MFG primed for a Gag specific response, which was demonstrated upon FIV challenge. All challenged cats became infected and no significant differences in viral loads were found between SL3261-MFG and SL3261-**CtxB** immunized cats.

L30 ANSWER 39 OF 71 CAPLUS COPYRIGHT 2006 ACS on STN
1997:470984 Document No. 127:157367 Gene fusion and expression of lipoprotein with **cholera toxin** B subunit and hepatitis B virus Pres2 epitope. Lin, Xu; Shi, Cheng-Hua; Cao, Cheng; Li, Ping; Bao, You-Di; Ma, Qing-Jun (Inst. Biotechnol., Acad. Military Med. Sci., Beijing, 100850, Peop. Rep. China). Shengwu Huaxue Zazhi, 13(3), 276-281 (Chinese) 1997. CODEN: SHZAE4. ISSN: 1000-8543. Publisher: Zhongguo Shengwu Huaxue Yu Fenzi Shengwu Xuehui.

AB Lipoprotein (lpp) of the outer membrane of Escherichia coli is a major protein of the cell wall. The N-terminal amino acids of lipoprotein and its artificial analogs were promising adjuvant in construction of peptide **vaccine** for their activity activating B-lymphocyte, macrophage and CTL. Nucleotides encoding signal peptides and N-terminal nine amino acids (LPP9) of lipoprotein were genetically fused to the 5' end of the **ctxB**-Pres2 gene by means of PCR. The fusion protein was well expressed with relatively low yield (about 0, 1-0.2 mg/l), and anchored in cell membrane. The fusion protein obtained in this way was more convenient and economical than chemical conjugation. The chimera was modified correctly and retained the biol. activity of both CTB and Pres2 as confirmed by 3H-palmitic acid labeling test and GM I Enzyme Linked Immunosorbent Assay resp. The signal peptide of lipoprotein was important to the correct modification of the chimera, and **ctxB** promoter was more efficient than that of lpp on the expression of chimers.

L30 ANSWER 40 OF 71 CAPLUS COPYRIGHT 2006 ACS on STN
1998:26759 Document No. 128:150086 Construction and characterization of versatile cloning vectors for efficient delivery of native foreign proteins to the periplasm of Escherichia coli. Jobling, Michael G.; Palmer, Leslie M.; Erbe, Jarrod L.; Holmes, Randall K. (Dep. Microbiol., Univ. Colorado Health Sci. Cent., Denver, CO, 80262, USA). Plasmid, 38(3), 158-173 (English) 1997. CODEN: PLSMDX. ISSN: 0147-619X. Publisher: Academic Press.

AB Induction of the wild type **cholera toxin** operon (ctxAB) from multicopy clones in Escherichia coli inhibited growth and resulted in low yields of **cholera toxin** (CT). We found that production of wild type CT or its B subunit (CT-B) as a periplasmic protein was toxic for E. coli, but by replacing the native signal sequences of both CT-A and CT-B with the signal sequence from the B subunit of E. coli heat-labile enterotoxin LTIIb we succeeded for the first time in producing CT holotoxin in high yield in E. coli. Based on these findings, we designed and constructed versatile cloning vectors that use the LTIIb-B signal sequence to direct recombinant native proteins with high efficiency to the periplasm of E. coli. We confirmed the usefulness of these vectors by producing two other secreted recombinant proteins. First, using phoA from E. coli, we demonstrated that alkaline phosphatase activity was 17-fold greater when the LTIIb-B signal sequence was used than when the native leader for alkaline phosphatase was used. Second, using the pspA gene that encodes pneumococcal surface protein A from Streptococcus pneumoniae, we produced a 299-residue amino-terminal fragment of PspA in E. coli in large amts. as a soluble periplasmic protein and showed that it was immunoreactive in Western blot with antibodies against native PspA. The vectors described here will be useful for further studies on structure-function relationships and **vaccine** development with CT and PspA, and they should be valuable as general tools for delivery of other secretion-competent recombinant proteins to the periplasm in E. coli.

L30 ANSWER 41 OF 71 CAPLUS COPYRIGHT 2006 ACS on STN
1997:431131 Document No. 127:120453 Immunogenicity of CT-B::DTx-B, CT-B::PT-S1*,S2, and CT-B::TT-B chimeric proteins: an approach to develop a safer DPT **vaccine**. Lu, Ying; Peterson, J.W.; Chopra, A.K. (Department of Microbiology and Immunology, The University of Texas Medical Branch, Galveston, TX, 77555-1019, USA). Vaccine Research, 6(1), 1-13 (English) 1997. CODEN: VAREES. ISSN: 1056-7909. Publisher: Liebert.

AB The authors report here the construction of several chimeric genes encoding the binding domains of diphtheria (DTx-B), pertussis (PT-S2), and tetanus (TT-B) toxins, as well as a modified PT-S1 enzymic subunit, which were placed downstream and in-frame with the immunomodulatory **cholera toxin** B-subunit (CT-B) gene. Each chimeric gene construct was hyperexpressed in Escherichia coli, and the fusion proteins, i.e., CT-B::DTx-B, CT-B::PT-S2, and CT-B::TT-B, reacted with antibodies to each component of the chimeras in an ELISA and Western blot anal. The hyperproduced proteins induced significant antibody titers in mice against both components of the chimeric proteins. A mutagenized PT-S1 subunit gene construct was made by first using site-directed mutagenesis to modify codons encoding amino acid residues Arg9 and Glu129, which are responsible for the ADP-ribosyltransferase activity of the PT-S1 subunit. The codons for these amino acids were replaced with those encoding Lys and Gly, resp., and the genetically inactivated PT-S1* gene was ligated downstream of the CT-B gene and expressed in E. coli. After hyperexpression, antibodies generated against the CT-B::PT-S1* construct neutralized the Chinese hamster ovary (CHO) cell clustering activity, which is a typical PT biol. response. Further, the antibodies blocked the elongation of CHO cells, which is a characteristic response of these cells to CT. These chimeric antigens may be beneficial in the development of alternative recombinant **vaccines** with minimal toxic side effects compared with those seen with the whole cell DPT **vaccine**.

L30 ANSWER 42 OF 71 CAPLUS COPYRIGHT 2006 ACS on STN
1996:597666 Document No. 125:239643 Genetic manipulation of Vibrio cholerae for **vaccine** development: construction of live attenuated El Tor candidate **vaccine** strains. Benitez, Jorge A.; Silva, Anisia J.; Rodriguez, Boris L.; Fando, Rafael; Campos, Javier; Robert, Alma; Garcia, Hilda; Garcia, Luis; Perez, Jose Luis; et al. (Grupo de Genetica del

Centro Nacional de Investigaciones Cientificas, Havana, Cuba). Archives of Medical Research, 27(3), 275-283 (English) 1996. CODEN: AEDEER. ISSN: 0188-4409. Publisher: Instituto Mexicano del Seguro Social.

AB The recent spread of El Tor cholera to America augments the need for an effective, safe and economical **vaccine**. In the present paper we describe the construction of live attenuated V. cholerae strains by specifically deleting the genes encoding **cholera toxin** and other putative toxins from the bacterial chromosome. To maximize the likelihood of exposing protective antigens relevant to currently circulating vibrios we selected for genetic manipulation recent epidemic V. cholerae isolates from Peru. The mutant strains did not produce **cholera toxin** in vitro and in vivo. Deletion of the virulence cassette was accompanied by marked attenuation in the infant mouse cholera model. A selected El Tor Ogawa candidate **vaccine** strain was refractory to acquisition of foreign genes by conjugation with toxigenic vibrios.

L30 ANSWER 43 OF 71 CAPLUS COPYRIGHT 2006 ACS on STN

1995:804342 Document No. 123:196581 Vibrio cholerae 01 (CVD111) and non-01 (CVD112 and CVD112rm) serogroup **vaccine** strains and methods of making same. Kaper, James B.; Levine, Myron M. (University of Maryland, USA). PCT Int. Appl. WO 9510300 A1 19950420, 109 pp. DESIGNATED STATES: W: AU, CA, CN, JP, KR, RU, US; RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE. (English). CODEN: PIXXD2. APPLICATION: WO 1994-US11424 19941007. PRIORITY: US 1993-133438 19931008; US 1993-133439 19931008.

AB Avirulent Vibrio cholerae strains of 01(CVD111) and non-01 (CVD112 and CVD112RM) serogroups having the DNA of the **cholera toxin** core and the RS1 sequences of the **cholera toxin** locus deleted, and further having a DNA encoding a resistance to mercury, and a DNA encoding the **cholera toxin** B subunit, or a part thereof sufficient to confer immunogenicity, re-inserted in the chromosome. Methods of making the avirulent V. cholerae 01 and non-01 strains of the invention, and cholera **vaccines** using these strains.

L30 ANSWER 44 OF 71 CAPLUS COPYRIGHT 2006 ACS on STN

1996:30105 Document No. 124:97719 Isolating restriction fragment deletions in Vibrio cholerae, and **vaccine** products with toxin gene deletions. Kaper, James B.; Baudry Maurelli, Bernadette; Fasano, Alessio (University of Maryland, USA). U.S. US 5470729 A 19951128, 55 pp. Cont.-in-part of U.S. Ser. No. 821,872, abandoned. (English). CODEN: USXXAM. APPLICATION: US 1992-931943 19920812. PRIORITY: US 1983-472276 19830304; US 1984-581406 19840217; US 1986-867633 19860527; US 1989-363383 19890605; US 1990-533315 19900605; US 1992-821872 19920116.

AB Methods of isolating deletion mutants of Vibrio cholerae are described. In one method, the deletion is predetd. by digestion with restriction endonucleases of known specificity. The deletions are inserted into the Vibrio cholerae chromosome by in vivo recombination between a plasmid carrying the desired deletion, with adjacent flanking sequences, and the Vibrio cholerae chromosome. In another method, an initial in vivo recombination event of homologous sequences from the recombinant plasmid into the chromosome provides a selectable marker at this site. A second in vivo recombination event between homologous flanking sequences results in excision of proficient genes from the chromosome with the end product being a deletion mutation. Also provided are methods for the isolation and characterization of a new Vibrio cholerae strain having a deletion in the ctx gene, as defined by AccI, XbaI, ClaI, and/or other restriction endonuclease sites and further having a deletion in the gene encoding zonula occludens toxin (zot). Thus, a culture of Vibrio cholerae was constructed with a deletion in the **cholera toxin** A and B subunit genes (ctxA and **ctxB**), which confers avirulence and retains the capacity to colonize the intestine of a host animal. A second

culture deletes the zonula occludens toxin gene (zot) in addition to the ctx genes, in order to reduce residual diarrhea in the host animal. Another culture has a region of chromosomal DNA coding for **cholera toxin** and zonula occludens toxin deleted, and having inserted a mercury resistance gene and DNA coding for B subunit of Vibrio toxin. These strains are avirulent without affecting other components necessary for immunity; they confer substantially close to 100% efficacy in humans against subsequent disease with a strain of a similar serotype and avoid undesirable side effects such as diarrhea and nausea and cramping. The ctx and zot gene deletions include the gene for ACE (accessory cholera enterotoxin), whose DNA sequence was determined

L30 ANSWER 45 OF 71 MEDLINE on STN DUPLICATE 20

96102052. PubMed ID: 8530395. Kinetics of acid-mediated disassembly of the B subunit pentamer of Escherichia coli heat-labile enterotoxin. Molecular basis of pH stability. Ruddock L W; Ruston S P; Kelly S M; Price N C; Freedman R B; Hirst T R. (Biological Laboratory, University of Kent, Canterbury, United Kingdom.) The Journal of biological chemistry, (1995 Dec 15) Vol. 270, No. 50, pp. 29953-8. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB The B-subunit pentamer of Escherichia coli heat-labile enterotoxin (EtxB) is highly stable, maintaining its quaternary structure in a range of conditions that would normally be expected to cause protein denaturation. In this paper the structural stability of EtxB has been studied as a function of pH by electrophoretic, immunochemical, and spectroscopic techniques. Disassembly of the cyclic pentameric structure of human EtxB occurs only below pH 2. As determined by changes in intrinsic fluorescence this process follows first-order kinetics, with the rate constant for disassembly being proportional to the square of the H+ ion concentration, and with an activation energy of 155 kJ mol-1. A C-terminal deletion mutant, hEtxB214, similarly shows first-order kinetics for disassembly but with a higher pH threshold, resulting in disassembly being seen at pH 3.4 and below. These findings are consistent with the rate-limiting step for disassembly of human EtxB being the simultaneous disruption of two interfaces by protonation of two C-terminal carboxylates. By comparison, disassembly of the B-subunit of **cholera toxin** (**CtxB**), a protein which shows 80% sequence identity with EtxB, exhibits a much lower stability to acid conditions; with disassembly of **CtxB** occurring below pH 3.9, with an activation energy of 81 kJ mol-1. Reasons for the observed differences in acid stability are discussed, and the implications of these findings to the development of oral **vaccines** using EtxB and **CtxB** are considered.

L30 ANSWER 46 OF 71 MEDLINE on STN DUPLICATE 21

95310029. PubMed ID: 7790086. Heterologous antigen expression in Vibrio cholerae vector strains. Butterton J R; Beattie D T; Gardel C L; Carroll P A; Hyman T; Killeen K P; Mekalanos J J; Calderwood S B. (Infectious Disease Unit, Massachusetts General Hospital, Boston 02114, USA.) Infection and immunity, (1995 Jul) Vol. 63, No. 7, pp. 2689-96. Journal code: 0246127. ISSN: 0019-9567. Pub. country: United States. Language: English.

AB Live attenuated vector strains of Vibrio cholerae were derived from Peru-2, a Peruvian El Tor Inaba strain deleted for the **cholera toxin** genetic element and attRS1 sequences, which was developed as a live, oral **vaccine** strain. A promoterless gene encoding the Shiga-like toxin I B subunit (slt-IB) was inserted in the V. cholerae virulence gene irgA by in vivo marker exchange, such that slt-IB was under transcriptional control of the iron-regulated irgA promoter. slt-IB was also placed under transcriptional control of the V. cholerae heat shock promoter, htpGp, and introduced into either the irgA or lacZ locus, or both loci, on the chromosome of Peru-2, generating JRB10, JRB11, or JRB12, respectively. A new technique was used to perform allelic exchange with

lacZ. This method uses plasmid p6891MCS, a pBR327 derivative containing cloned V. cholerae lacZ, to insert markers of interest into the V. cholerae chromosome. Recombinants can be detected by simple color screening and antibiotic selection. In vitro measurements of Slt-IB produced by the vector strains suggested that expression of Slt-IB from the irgA and htpG promoters was synergistic and that two copies of the gene for Slt-IB increased expression over a single copy. The V. cholerae vectors colonized the gastrointestinal mucosa of rabbits after oral immunization, as demonstrated by very high serum antibody responses to V. cholerae antigens. Comparison of the serologic responses to the B subunit of **cholera toxin** (**CtxB**) following orogastric inoculation either with the wild-type C6709 or with Peru-10, a strain containing **ctxB** regulated by htpGp, suggested that both the **cholera toxin** and heat shock promoters were active in vivo, provoking comparable immunologic responses. Orogastric inoculation of rabbits with vector strains evoked serum immunoglobulin G (IgG) responses to Slt-IB in two of the four strains tested; all four strains produced biliary IgA responses. No correlation was observed between the type of promoter expressing slt-IB and the level of serum IgG or biliary IgA response, but the vector strain containing two copies of the gene for slt-IB evoked greater serum IgG responses than strains containing a single copy, consistent with the increased expression of Slt-IB from this strain observed in vitro. A comparison of the serum and biliary antibody responses to Slt-IB expressed from htpGp versus **CtxB** expressed from the same promoter suggested that **CtxB** is a more effective orally delivered immunogen.

L30 ANSWER 47 OF 71 MEDLINE on STN DUPLICATE 22

95197259. PubMed ID: 7890393. Oral immunization with the dodecapeptide repeat of the serine-rich Entamoeba histolytica protein (SREHP) fused to the **cholera toxin** B subunit induces a mucosal and systemic anti-SREHP antibody response. Zhang T; Li E; Stanley S L Jr. (Department of Medicine, Washington University School of Medicine, St. Louis, Missouri 63110.) Infection and immunity, (1995 Apr) Vol. 63, No. 4, pp. 1349-55. Journal code: 0246127. ISSN: 0019-9567. Pub. country: United States. Language: English.

AB The intestinal protozoan parasite Entamoeba histolytica causes amebic dysentery, a major cause of morbidity worldwide. The induction of a mucosal antibody response capable of blocking amebic adhesion to intestinal cells could represent an approach to preventing E. histolytica infection and disease. Here we describe the expression of a chimeric protein containing an immunogenic dodecapeptide derived from the serine-rich E. histolytica protein (SREHP), fused to the **cholera toxin** B subunit (**CtxB**). The **CtxB**-SREHP-12 chimeric protein was purified from Escherichia coli lysates and retained the critical GM1 ganglioside-binding activity of the **CtxB** moiety. Mice fed the **CtxB**-SREHP-12 fusion protein along with a subclinical dose of **cholera toxin** developed mucosal immunoglobulin A and immunoglobulin G and systemic antibody responses that recognized recombinant and native SREHP. Our study confirms the feasibility of inducing mucosal immune responses to immunogenic peptides by their genetic fusion to the **CtxB** subunit and identifies the **CtxB**-SREHP-12 chimeric protein as a candidate oral **vaccine** to prevent E. histolytica infection.

L30 ANSWER 48 OF 71 SCISEARCH COPYRIGHT (c) 2006 The Thomson Corporation on STN

1995:511427 The Genuine Article (R) Number: RL566. IMMUNOREGULATORY ROLE OF H-2 AND INTRA-H-2 ALLELES ON ANTIBODY-RESPONSES TO RECOMBINANT PREPARATIONS OF B-SUBUNITS OF ESCHERICHIA-COLI HEAT-LABILE ENTEROTOXIN (RETXB) AND **CHOLERA-TOXIN** (RCTXB). NASHAR T O (Reprint); HIRST T R. UNIV KENT, RES SCH BIOSCI, CANTERBURY CT2 7NJ, KENT, ENGLAND (Reprint). VACCINE (JUN 1995) Vol. 13, No. 9, pp. 803-810. ISSN:

0264-410X. Publisher: BUTTERWORTH-HEINEMANN LTD, LINACRE HOUSE JORDAN HILL, OXFORD, OXON, ENGLAND OX2 8DP. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The immunoregulatory role of H-2 and intra-H-2 alleles on antibody responses to recombinant preparations of B-subunits of Escherichia coli heat-labile enterotoxin (rEtxB) and **cholera toxin** (rCtxB) is reported. Oral delivery of rEtxB to congenic mice of several different H-2 haplotypes resulted in H-2 dependent serum IgG responses (H-2(d) > H-2(b) = H-2(q) > H-2(a) > H-2(k)) and a similar spectrum of intestinal IgA responses in those strains tested Responses to rEtxB and rCtxB were found to be differentially modulated by the H-2 locus, with significant differential effects in H-2(b) and H-2(d) congenic strains (H-2(d) > H-2(b) for rEtxB; H-2(b) > H-2(d) for rCtxB), Additionally, it was found that when rEtxB was fed to mice previously primed (orally) with either rEtxB or rCtxB only those mice primed with rEtxB exhibited a booster response. A second booster immunisation with rEtxB in rCtxB-primed mice produced an H-2 dependent spectrum of responses characteristic of those elicited by rEtxB, with the antibodies predominantly directed against rEtxB and not rCtxB. These results indicate that the differential response to rEtxB and rCtxB is set at the T- and B-cell level. Also, immunoregulation of antibody responses to rEtxB by intra-H-2 I-E in mice transgenic for the entire IE(a)(k) gene was investigated. No significant difference between responses in transgene-positive and -negative mice was found, suggesting that antigen presentation does not involve I-E, but occurs in the context of I-A. The implications of these results for the design of **vaccines** against enterotoxigenic E. coli and cholera diarrhoea are discussed.

L30 ANSWER 49 OF 71 CAPLUS COPYRIGHT 2006 ACS on STN
1995:227412 Document No. 122:48495 Membrane expression of heterologous genes with plasmids containing export signals from Salmonella typhimurium. Niesel, David W.; Moncrief, J. Scott; Phillips, Linda H. (Board of Regents, The University of Texas, USA). U.S. US 5356797 A 19941018, 26 pp. (English). CODEN: USXXAM. APPLICATION: US 1991-792525 19911115.

AB The invention relates to nucleic acid segments useful in the construction of expression vectors for expression of heterologous polypeptides directed to particular areas of the host cell. Selected constructs (e.g., the plasmid pZIP-OUT) direct production of polypeptides to the outer membrane surface of the cell. Other constructs (e.g., plasmid pZIP-IN) direct expression of heterologous polypeptides to the inner membrane/periplasm of the host cell. Isolation of the Salmonella typhimurium DNA segments was accomplished by isolation of DNA fragments containing phoA gene fusions resulting from random transposition of TnphoA (a Tn4 derivative encoding Escherichia coli alkaline phosphatase minus the signal sequence and expression signals, inserted into the left IS50L element). Plasmids containing phoA gene fusions can then be used as exposition vectors. The SSP1 and the PvuII restriction sites in phoA are blunt ended sites at which inframe insertions of a gene of interest can be inserted. The resulting tribrid gene fusions contain the expression and export signals of the target gene fused inframe with the phoA and gene of interest. The system is demonstrated by fusions with the **cholera toxin** B subunit gene **ctxB** and a 60-kDa fragment of HIV gp120. Transformed host cells are potentially useful for the production of **vaccines** or immunogens elicited in response to antigens expressed on the outer membranes of the host cells.

L30 ANSWER 50 OF 71 CAPLUS COPYRIGHT 2006 ACS on STN
1996:359780 Document No. 125:28185 **Cholera toxin** A1-chain analogs which retain immunogenicity but lack ADP-ribosyltransferase activity. Burnette, Walter Neal; Kaslow, Harvey Robin (Amgen Inc., USA; University of Southern California). Indian IN 173616 A 19940611, 74 pp. (English). CODEN: INXXAP. APPLICATION: IN 1992-MA262 19920504.

AB Analogs of the catalytic subunit of **cholera toxin** are produced by of cultivating prokaryotic or eukaryotic host cells transformed and transfected with DNA vectors having DNA sequences encoding the analogs isolating the desired polypeptides in a known manner. The development of subunits and subunit analogs of the **cholera toxin** by recombinant DNA techniques provides **vaccine** products that can retain their biol. activity and immunogenicity, and can confer protection against disease challenge. The genetically engineered modifications of the subunits result in products that retain immunogenicity, yet are reduced in, or are essentially free of enzymic activity associated with toxin reactogenicity. Thus, recombinant **cholera toxin** A1 chains (CTXA1) were synthesized in Escherichia coli under control of an optimized expression vector by standard methods. N-methionyl-B-chain was also cloned and expressed to prepare holo-toxins with full immunogenicity. An oligonucleotide linker substituted an initiating methionine codon for the signal peptide-encoded sequence of the preproA-chain-encoding DNA; this N-terminal methionyl residue is probably not processed away in the final products. A1-chain analogs were produced by site-specific mutagenesis, and the ADP ribosyltransferase activity tested for autocatalysis, for Gsα protein, and for H27 fibroblast and erythrocyte membranes. Mutagenesis of the amino acid residues at positions Arg7, His44, His70, Glu112, and Asp9, and truncation of the C-terminus (at Trp179 of the mature native CTXA sequence) resulted in diminished or essentially no ADP-ribosyltransferase activity.

L30 ANSWER 51 OF 71 MEDLINE on STN DUPLICATE 23

94314415. PubMed ID: 8039872. Construction and characterization of recombinant Vibrio cholerae strains producing inactive **cholera toxin** analogs. Hase C C; Thai L S; Boesman-Finkelstein M; Mar V L; Burnette W N; Kaslow H R; Stevens L A; Moss J; Finkelstein R A. (Department of Molecular Microbiology and Immunology, School of Medicine, University of Missouri, Columbia 65212.) Infection and immunity, (1994 Aug) Vol. 62, No. 8, pp. 3051-7. Journal code: 0246127. ISSN: 0019-9567. Pub. country: United States. Language: English.

AB The catalytic A subunit of **cholera toxin** (CT-A) is capable of ADP-ribosylating the guanine nucleotide-binding protein, which regulates cell adenylyl cyclase, leading to the life-threatening diarrhea of cholera. Amino acids involved in the enzymatic activity of CT-A have previously been identified. By means of site-directed mutagenesis, an analog of the CT-A subunit gene was created with codon substitutions for both Arg-7 and Glu-112, each of which has been shown to produce subunits lacking ADP-ribosyltransferase activity. The mutated gene fragment was exchanged for the wild-type copy in the previously cloned ctxAB operon from El Tor biotype, Ogawa serotype Vibrio cholerae strain 3083, which produces CT-2. Further, the zonula occludens toxin gene, zot, was inactivated by an insertional mutation to create the new plasmid construct pCT-2*. Additionally, a DNA fragment encoding the B subunit of CT-1 (CT produced by classical biotype, Inaba serotype V. cholerae strain 569B) was exchanged for the homologous part in pCT-2*, resulting in the creation of pCT-1*. These plasmid constructs were introduced into the CT-negative V. cholerae mutant strain JBK70 (E1 Tor biotype, Inaba serotype); CT-A-B+ derivatives CVD101 and CVD103 of classical biotype Ogawa and Inaba serotype strains 395 and 569B, respectively; El Tor biotype Inaba and Ogawa serotype strains C6706 and C7258, respectively, recently isolated in Peru; and O139 (synonym Bengal) strain SG25-1 from the current epidemic in India. Recombinant toxins (CT-1* and CT-2*), partially purified from culture supernatants of transformed JBK70, were shown to be inactive on mouse Y1 adrenal tumor cells and in an in vitro ADP-ribosyltransferase assay. CT-1* and CT-2* reacted with polyclonal and monoclonal antibodies against both A and B subunits of CT. The toxin analogs reacted with antibodies against CT-A and CT-B on cellulose acetate strips and in a GM1 enzyme-linked immunosorbent assay; they reacted appropriately with

B-subunit epitype-specific monoclonal antibodies in checkerboard immunoblots, and they formed precipitin bands with GM1-ganglioside in Ouchterlony tests. However, the reactions of the modified proteins with anti-A-subunit monoclonal antibodies were weaker than the reactions with wild-type holotoxins. V, cholerae strains carrying ctxA*, with either **ctxB**-1 or **ctxB**-2, and inactivated zot genes were created by homologous recombination. The recombinant strains and the purified toxin analogs were inactive in the infant rabbit animal model.(ABSTRACT TRUNCATED AT 400 WORDS)

L30 ANSWER 52 OF 71 MEDLINE on STN DUPLICATE 24

95066317. PubMed ID: 7975840. Construction and evaluation of an expression vector allowing the stable expression of foreign antigens in a Salmonella typhimurium **vaccine** strain. Tijhaar E J; Zheng-Xin Y; Karlas J A; Meyer T F; Stukart M J; Osterhaus A D; Mooi F R. (Laboratory of Immunobiology, National Institute of Public Health and Environmental Protection, Bilthoven, The Netherlands.) Vaccine, (1994 Aug) Vol. 12, No. 11, pp. 1004-11. Journal code: 8406899. ISSN: 0264-410X. Pub. country: ENGLAND: United Kingdom. Language: English.

AB Salmonella strains have great potential as live carriers of heterologous antigens to induce immunity against a variety of infectious diseases. However, the amount of heterologous antigen required to induce an adequate immune response may be toxic for the bacterium and result in cell death, overattenuation or loss of expression of the heterologous antigen. To solve this problem an expression vector was developed with a strong promoter located on a DNA fragment which is inverted at random. Antigen is only expressed in one particular orientation of the promoter. Thus a bacterial population harbouring the plasmid will consist of a subpopulation which does not produce heterologous antigen, and is therefore not affected in growth, persistence and dissemination within the host. Further, this non-producing population will continuously segregate antigen-producing bacteria. To evaluate the system, **CtxB** was used as a model antigen. Analysis of the plasmid DNA isolated from Salmonella revealed a selection against the promoter orientation that directs transcription of the **ctxB** gene. In spite of this, the vector was stably maintained in vivo and induced **CtxB**-specific IgA and IgG in mice. These results indicate that this kind of expression vector may offer a solution to the problem of unstable expression of foreign antigens in live bacterial **vaccine** strains.

L30 ANSWER 53 OF 71 CAPLUS COPYRIGHT 2006 ACS on STN

1993:464935 Document No. 119:64935 Membrane targeting of heterologous proteins in bacteria and use of the bacteria or membranes as **vaccines**. Niesel, David W.; Moncrief, J. Scott; Phillips, Linda H. (University of Texas System, USA). PCT Int. Appl. WO 9310246 A1 19930527, 73 pp. DESIGNATED STATES: W: AT, AU, BB, BG, BR, CA, CH, CS, DE, DK, ES, FI, GB, HU, JP, KP, KR, LK, LU, MG, MN, MW, NL, NO, PL, RO, RU, SD, SE, UA; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE, DK, ES, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NL, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1992-US9659 19921112. PRIORITY: US 1991-792252 19911115.

AB A method for producing heterologous proteins, especially antigens, in bacterial cells and exporting them to the inner membrane/periplasm or the outer membrane surface is described. Bacterial cells, or membranes of these cells, containing these heterologous proteins may be used as **vaccines**. Plasmids pZIP-IN and pZIP-OUT, which contain protein-encoding Salmonella typhimurium genomic DNA fused to the Escherichia coli phoA gene, direct the fusion protein to the inner and outer membranes, resp. A pZIP-OUT derivative, in which a **ctxB** gene fragment was fused to the phoA sequence, was prepared The chimeric gene, encoding a tripartite S. typhimurium protein-PhoA-**CtxB** fusion, was expressed in an attenuated Salmonella strain. The **cholera toxin** B subunit peptide was immunol. localized to the outer surface membrane.

L30 ANSWER 54 OF 71 MEDLINE on STN DUPLICATE 25

94011341. PubMed ID: 8406837. CVD110, an attenuated Vibrio cholerae O1 El Tor live oral **vaccine** strain. Michalski J; Galen J E; Fasano A; Kaper J B. (Department of Medicine, University of Maryland School of Medicine, Baltimore 21201.) Infection and immunity, (1993 Oct) Vol. 61, No. 10, pp. 4462-8. Journal code: 0246127. ISSN: 0019-9567. Pub. country: United States. Language: English.

AB The recent expansion of the seventh cholera pandemic into South America emphasizes the need for a safe, long-lasting, protective, and nonreactogenic **vaccine** for this disease. Since the predominant Vibrio cholerae O1 strains in the world today are of the El Tor biotype, a bivalent **vaccine** containing both classical and El Tor biotypes may be desirable. We have constructed a new oral **vaccine** candidate, V. cholerae CVD110 El Tor, Ogawa, from which all toxin genes so far identified in V. cholerae have been deleted. Three of these genes, those encoding **cholera toxin** (ctx), zonula occludens toxin (zot), and accessory cholera enterotoxin (ace), are located on a 4.5-kb virulence cassette flanked by repetitive sequences (RS1 elements). Homologous recombination between these RS1 elements resulted in the deletion of this virulence cassette to yield V. cholerae CVD109. Insertion of genes encoding mercury resistance (mer) and the **cholera toxin** B subunit (**ctxB**) into the hemolysin locus (hlyA) produced CVD110. This insertion serves three purpose. (i) It genetically tags the **vaccine** strain so as to distinguish it from wild-type V. cholerae O1. (ii) It produces **cholera toxin** B subunit in order to elicit antitoxic immunity. (iii) It inactivates the hemolysin gene, rendering the strain nonhemolytic on sheep erythrocyte plates. Supernatants from V. cholerae CVD110 cultures are nonreactogenic when assayed in Ussing chambers.

L30 ANSWER 55 OF 71 MEDLINE on STN

93234575. PubMed ID: 8475125. CTX genetic element encodes a site-specific recombination system and an intestinal colonization factor. Pearson G D; Woods A; Chiang S L; Mekalanos J J. (Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, MA 02115.) Proceedings of the National Academy of Sciences of the United States of America, (1993 Apr 15) Vol. 90, No. 8, pp. 3750-4. Journal code: 7505876. ISSN: 0027-8424. Pub. country: United States. Language: English.

AB In Vibrio cholerae, the genes encoding **cholera toxin** (ctxAB) are located on a segment of DNA (termed the "core" region) that is flanked by two or more copies of a repeated sequence called RS1. Together these DNA units comprise the CTX genetic element. Evidence presented here suggests that RS1 sequences encode a site-specific recombination system, which allows integration of a suicide plasmid carrying RS1 into an 18-base-pair sequence (attRS1) located on the chromosome of nontoxigenic V. cholerae strains. Strains of V. cholerae with large deletions removing attRS1 and the entire CTX genetic element no longer undergo site-specific recombination with the RS1 sequence. Additionally, these deletion strains show a defect in intestinal colonization. Recombination experiments localize the gene responsible for enhancing colonization to a portion of the core region of the CTX element. The identified gene encodes a peptide that is highly similar in amino acid sequence to the flexible pilin of Aeromonas hydrophila. These results have important implications in the construction of stable, live attenuated cholera **vaccines**.

L30 ANSWER 56 OF 71 MEDLINE on STN

94059512. PubMed ID: 7764248. Large-scale production of Vibrio cholerae toxin B subunit for use in oral **vaccines**. Lebens M; Johansson S; Osek J; Lindblad M; Holmgren J. (University of Goteborg, Dept. of Medical Microbiology and Immunology, Sweden.) Bio/technology (Nature Publishing Company), (1993 Dec) Vol. 11, No. 13, pp. 1574-8. Journal code: 8309273. ISSN: 0733-222X. Pub. country: United States. Language: English.

AB By systematically manipulating promoter and ribosome binding structures, plasmid copy number and the structure of the **cholera toxin** B (CTB) subunit gene, we were able to develop a plasmid expression system that, when used in conjunction with an optimized growth medium, provided yields of CTB approaching one gram per liter. The CTB protein which was secreted to > 95%, could readily be purified from the growth medium of a V. cholerae production strain and was shown to be immunologically indistinguishable from previously used **vaccine** preparations of native or recombinant CTB.

L30 ANSWER 57 OF 71 MEDLINE on STN DUPLICATE 26
93138755. PubMed ID: 8423068. Reduction in oral immunogenicity of **cholera toxin** B subunit by N-terminal peptide addition. Dertzbaugh M T; Elson C O. (Division of Gastroenterology, School of Medicine, University of Alabama, Birmingham 35294.) Infection and immunity, (1993 Feb) Vol. 61, No. 2, pp. 384-90. Journal code: 0246127. ISSN: 0019-9567. Pub. country: United States. Language: English.

AB The mucosal adjuvanticity of **cholera toxin** and the potential of the B subunit of **cholera toxin** (**CtxB**) to serve as an oral **vaccine** carrier have prompted interest in the coupling of immunogenic peptides to this protein. The purpose of this study was to determine how such fusions affect the function of **CtxB**. Oligonucleotides were genetically fused to the 5' terminus of the **ctxB** gene to encode additional amino acids of 8, 12, and 24 residues in length. None of these additions affected the ability of **CtxB** to oligomerize, as determined by nondenaturing sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Circular dichroism revealed no difference in conformation between the modified B subunits, regardless of the length of the addition. However, when compared with native **CtxB**, additions to the N terminus induced a consistent change in the net conformation of the protein. By using a competitive enzyme immunoassay, the affinity of the modified B subunits for GM1 ganglioside was shown to gradually decrease with increasing length of the N-terminal addition. A similar pattern was observed for the ability of the chimeras to inhibit proliferation of concanavalin A-stimulated spleen cells in vitro, which is a previously described functional property of **CtxB** that is dependent on its binding to cells. Lastly, the oral immunogenicity of these chimeras was found to be less than that of native **CtxB**. These results indicate that large fusions to the N terminus of **CtxB** can significantly affect its biological properties and could reduce its value as a mechanism for effective mucosal immunization.

L30 ANSWER 58 OF 71 MEDLINE on STN
93175126. PubMed ID: 7679865. Current progress in the development of the B subunits of **cholera toxin** and Escherichia coli heat-labile enterotoxin as carriers for the oral delivery of heterologous antigens and epitopes. Nashar T O; Amin T; Marcello A; Hirst T R. (Biological Laboratory, University of Kent, Canterbury, UK.) Vaccine, (1993) Vol. 11, No. 2, pp. 235-40. Ref: 49. Journal code: 8406899. ISSN: 0264-410X. Pub. country: ENGLAND: United Kingdom. Language: English.

AB The development of non-living carrier systems for delivery of protective antigens or epitopes to the immune system represents both a fundamental and an applied aspect of vaccinology. A wide range of carrier systems, ranging from inert supports to proteins that exert direct immunomodulating effects on the immune response, are being studied. In this overview we describe the current progress in the development of the B-subunits of **cholera toxin** and Escherichia coli heat-labile enterotoxin as potential protein carriers for the oral delivery of chemically and genetically attached antigens and epitopes.

L30 ANSWER 59 OF 71 MEDLINE on STN DUPLICATE 27
93114904. PubMed ID: 8418065. Comparative effectiveness of the

cholera toxin B subunit and alkaline phosphatase as carriers for oral **vaccines**. Dertzbaugh M T; Elson C O. (Division of Gastroenterology, School of Medicine, University of Alabama, Birmingham 35294.) Infection and immunity, (1993 Jan) Vol. 61, No. 1, pp. 48-55. Journal code: 0246127. ISSN: 0019-9567. Pub. country: United States. Language: English.

AB The purpose of this study was to determine whether the B subunit of **cholera toxin** (**CtxB**) has adjuvant activity over and above serving as a carrier protein for orally administered **vaccines**. An oligonucleotide that encodes an antigenic determinant (GtfB.1) from the glucosyltransferase B gene (gtfB) of Streptococcus mutans was genetically fused to the 5' terminus of either the **CtxB** gene (**ctxB**) or the Escherichia coli alkaline phosphatase gene (phoA). The resulting chimeric proteins were expressed in a phoA mutant strain of E. coli and then purified. The antigenicities of the proteins were confirmed by immunoblotting analysis using antisera specific for GtfB, **CtxB**, or PhoA. An equimolar amount of peptide on each carrier was administered by gastric intubation to mice three times at 10-day intervals. Antibody titers to the peptide, **CtxB**, and PhoA (in the serum, intestine, vagina, saliva, and bronchus) were determined by enzyme immunoassay. Antibody to the peptide was detected only in the sera of mice immunized with the peptide fused to **CtxB**. No antipeptide antibody was detected in mice immunized with the peptide fused to PhoA. The lack of detectable levels of antipeptide antibody in intestinal lavage fluid was attributed to dilution of the sample beyond the sensitivity of the assay. This was confirmed by cultivation of Peyer's patch and mesenteric lymph node tissue from mice orally immunized with the GtfB.1::**CtxB** chimera. Using this method, antipeptide antibody was detected in the culture fluid. We conclude that **CtxB** possesses unique properties that allow it to act as more than a simple carrier protein.

L30 ANSWER 60 OF 71 MEDLINE on STN DUPLICATE 28

93107263. PubMed ID: 7678018. Use of automated sequencing of polymerase chain reaction-generated amplicons to identify three types of **cholera toxin** subunit B in Vibrio cholerae O1 strains. Olsvik O; Wahlberg J; Petterson B; Uhlen M; Popovic T; Wachsmuth I K; Fields P I. (Division of Bacterial and Mycotic Diseases, Centers for Disease Control, Atlanta, Georgia 30333.) Journal of clinical microbiology, (1993 Jan) Vol. 31, No. 1, pp. 22-5. Journal code: 7505564. ISSN: 0095-1137. Pub. country: United States. Language: English.

AB **Cholera toxin** is the principal factor causing the profuse intestinal fluid secretion that is characteristic of cholera. The DNA sequences of the **cholera toxin** subunit B structural genes from 45 Vibrio cholerae O1 strains isolated in 29 countries over a period of 70 years were determined by automated DNA sequencing of polymerase chain reaction-generated amplicons. Three types of **cholera toxin** B subunit gene (**ctxB**) were identified. Genotype 1 was found in strains of classical biotype worldwide and El Tor biotype strains associated with the U.S. Gulf Coast, genotype 2 was found in El Tor biotype strains from Australia, and genotype 3 was found in El Tor biotype strains from the seventh pandemic and the recent Latin American epidemic. All base changes correspond to an amino acid substitution in the B subunit of the **cholera toxin**. Heterogenicity in the B subunit could have implications for **vaccine** development and diagnostic tests for **cholera toxin** and antitoxin. We conclude that this technology provides timely and potentially useful epidemiological information.

L30 ANSWER 61 OF 71 CAPLUS COPYRIGHT 2006 ACS on STN

1993:58102 Document No. 118:58102 Preparation of immunoprotective antigens of pneumococcal surface protein A. Briles, David E.; Yother, Janet L.; McDaniel, Larry S. (UAB Research Foundation, USA). PCT Int. Appl. WO

9214488 A1 19920903, 44 pp. DESIGNATED STATES: W: AU, CA, FI, JP, NO, US; RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LU, MC, NL, SE. (English). CODEN: PIXXD2. APPLICATION: WO 1992-US857 19920212. PRIORITY: US 1991-656773 19910215.

AB A purified, truncated pneumococcal surface protein A (PspA) presenting epitopes is prepared with a mutant of Streptococcus pneumoniae for use in **vaccines**. This antigen is useful because it is independent of pneumococcal capsular type. The truncated PspA lacks at least the functional cell membrane anchor domain, or the repeat region and the proline-rich regions. A 43-kDa (apparent mol. weight) truncated PspA was secreted by S. pneumoniae Rx1, a novel mutant prepared by insertional duplication mutagenesis. This truncated PspA gene can be expressed in Mycobacterium or Escherichia coli, e.g. as a fusion protein with the B-unit of **cholera toxin** (CTB), for preparation of **vaccine** against pneumococcal infection.

L30 ANSWER 62 OF 71 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN

1992:270440 Document No.: PREV199242129390; BR42:129390. COMPARISON OF THE ORAL IMMUNOGENICITY OF A FOREIGN PEPTIDE WHEN COUPLED TO **CHOLERA TOXIN** B SUBUNIT OR TO ALKALINE PHOSPHATASE. DERTZBZUGH M T [Reprint author]; ELSON C O. UNIV ALABAMA BIRMINGHAM, BIRMINGHAM, ALA 35294, USA. FASEB Journal, (1992) Vol. 6, No. 4, pp. A1229. Meeting Info.: MEETING OF THE FEDERATION OF AMERICAN SOCIETIES FOR EXPERIMENTAL BIOLOGY (FASEB), PART 1, ANAHEIM, CALIFORNIA, USA, APRIL 5-9, 1992. FASEB (FED AM SOC EXP BIOL) J. CODEN: FAJOEC. ISSN: 0892-6638. Language: ENGLISH.

L30 ANSWER 63 OF 71 MEDLINE on STN

93110970. PubMed ID: 1361701. Molecular design of cholera **vaccines** . Manning P A. (Department of Microbiology and Immunology, University of Adelaide, South Australia.) Vaccine, (1992) Vol. 10, No. 14, pp. 1015-21. Ref: 64. Journal code: 8406899. ISSN: 0264-410X. Pub. country: ENGLAND: United Kingdom. Language: English.

AB Cholera is still a serious public health problem in developing countries, particularly those in tropical regions. This has stimulated considerable research into the molecular analysis of pathogenesis resulting in the identification of a number of critical components required for both colonization of the gut mucosa and the disease symptoms. These components are the targets for rational molecular approaches to **vaccine** development.

L30 ANSWER 64 OF 71 CAPLUS COPYRIGHT 2006 ACS on STN

1993:574963 Document No. 119:174963 Vibrio cholerae toxin B subunit gene expressed in a Salmonella **vaccine** strain. Tian, Jinghui; Lu, Deru (2nd Mil. Med. Univ., Shanghai, 200433, Peop. Rep. China). Weishengwu Xuebao, 32(5), 320-7 (Chinese) 1992. CODEN: WSHPA8. ISSN: 0001-6209.

AB The V. cholerae toxin B subunit (**ctxB**)gene was inserted into pYA248 plasmid with the aspartate β-semialdehyde dehydrogenase (asd) gene and the recombinant plasmid was transformed into Salmonella typhimurium without asd gene. The ctx B gene was highly expressed and its product secreted into the medium. This strain was able to colonize in the intestinal epithelium. Oral immunity and general immunity could produce antibodies at high level and enhance cellular immune responses. The animals orally inoculated with S. typhimurium + 4072 (pYA-ctx B) **vaccine** had remarkable protection against virulent V. cholerae and S. typhimurium.

L30 ANSWER 65 OF 71 CAPLUS COPYRIGHT 2006 ACS on STN

1992:100630 Document No. 116:100630 Avirulent Vibrio cholerae strains and method for production of same by directed deletion of genomic material. Kaper, James B.; Baudry-Maurelli, Bernadette; Fasano, Alessio (University

of Maryland, USA). PCT Int. Appl. WO 9118979 A1 19911212, 82 pp. DESIGNATED STATES: W: AU, CA, JP, SU; RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LU, NL, SE. (English). CODEN: PIXXD2. APPLICATION: WO 1991-US3812 19910605. PRIORITY: US 1990-533315 19900605.

AB V. cholerae of the Ogawa or Inaba serotype having deletions in the toxin and in the zonula occludens toxin genes are described. Methods for forcing directed deletions in Vibrio cholerae to cause attenuation of virulence without loss of ability to colonize the intestine are described. The resulting bacteria are suitable for use in live oral **vaccines**. The method uses a plasmid carrying a region derived from one of the virulence genes that is interrupted and a selectable marker. This is introduced into the virulent host by standard mating where it usually integrates into the host chromosome. The plasmid-carrying host is then mated with a strain carrying a second plasmid that is incompatible with the first. The mating is then selected for bacteria carrying the second selectable markers only; this will only be possible when the first marker has been excised from the chromosome by homologous recombination. The recombination also results in deletion of a region of the chromosome carried by the plasmid. Plasmids were constructed for use in the disruption of the genes for **cholera toxin** and zona occludens toxin and their uses is demonstrated.

L30 ANSWER 66 OF 71 CAPLUS COPYRIGHT 2006 ACS on STN

1991:576713 Document No. 115:176713 Recombinant **cholera toxin**-antigenic peptide fusion proteins and their use as **vaccines**. Dertzbaugh, Mark T.; Macrina, Francis L. (Center for Innovative Technology, USA). PCT Int. Appl. WO 9107979 A1 19910613, 68 pp. DESIGNATED STATES: W: CA, JP; RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LU, NL, SE. (English). CODEN: PIXXD2. APPLICATION: WO 1990-US6811 19901128. PRIORITY: US 1989-442783 19891129.

AB Recombinant fusion proteins comprising an antigen epitope linked to the N-terminus of a **cholera toxin** B subunit fragment are produced. These may be used as **vaccines**. A chimeric gene containing the ompA signal sequence, a fragment of the glucosyltransferase B gene (gtfB) of Streptococcus mutans, and a part of the **ctxB** gene was constructed and expressed in Escherichia coli. Antisera to the fusion protein produced by these transformants inhibited the S. mutans enzyme in vitro. This recombinant protein is proposed as a **vaccine** against dental caries.

L30 ANSWER 67 OF 71 MEDLINE on STN DUPLICATE 29

90316661. PubMed ID: 2370100. Oral immunization of mice with a live recombinant Yersinia enterocolitica O:9 strain that produces the **cholera toxin** B subunit. Sory M P; Hermand P; Vaerman J P; Cornelis G R. (Unite de Microbiologie, Universite Catholique de Louvain, Belgium.) Infection and immunity, (1990 Aug) Vol. 58, No. 8, pp. 2420-8. Journal code: 0246127. ISSN: 0019-9567. Pub. country: United States. Language: English.

AB The 70-kilobase pYV plasmid of Yersinia enterocolitica encodes a set of proteins called Yops that are produced during infection. To use Y. enterocolitica as a live carrier to present the **cholera toxin** B (CT-B) subunit to the immune system, we constructed an operon fusion between **ctxB** and the yop51 gene. This operon fusion was either cloned on an RSF1010-derived plasmid or integrated into the pYV plasmid itself. In Y. enterocolitica, both constructions directed the synthesis of free CT-B only under conditions of Yops production, i.e., at 37 degrees C in a medium deprived of Ca2+. Bacteria containing both types of recombinant plasmids were given orally to mice. A serum antibody response against CT-B was detected in both cases. A secretory immunoglobulin A activity specific to CT-B was also observed in the intestinal secretions. According to immunoblot analysis, the serum antibody response was only directed against the polymeric form of the B subunit. The **ctxB** gene was also inserted in frame within yop51,

giving a chimeric Yop51-CT-B protein that was secreted into the surrounding medium. In this case, however, no antibody response was observed after oral inoculation of mice. This lack of response probably results from the inability of the hybrid protein to assemble into the polymeric form of the B subunit.

L30 ANSWER 68 OF 71 MEDLINE on STN DUPLICATE 30
91326975. PubMed ID: 2101483. Delivery of the **cholera toxin** B subunit by using a recombinant Yersinia enterocolitica strain as a live oral carrier. Sory M P; Cornelis G R. (Unite de Microbiologie, Universite Catholique de Louvain, Brussels.) Research in microbiology, (1990 Sep-Oct) Vol. 141, No. 7-8, pp. 921-9. Journal code: 8907468. ISSN: 0923-2508. Pub. country: France. Language: English.

AB The gene **ctxB** encoding the **cholera toxin** B subunit was subcloned to design its production by Yersinia enterocolitica. It was joined in two ways to yopH, a gene of the virulence plasmid pYV specific to this genus. This gene encodes one of the major Yop proteins (YopH) secreted by bacteria incubated at 37 degrees C in a Ca(2+)-deprived medium. In a first construction, an operon fusion was obtained between **ctxB** and yopH so that CT-B and a truncated YopH protein were produced. The recombinant CT-B from Y. enterocolitica was structurally and antigenically similar to CT-B produced by Vibrio cholerae. In another construction, the fusion gene obtained directed the production of YopH'/CT-B hybrid proteins that were secreted by Y. enterocolitica. In both cases, Y. enterocolitica directed the production of the recombinant proteins only when the bacteria were incubated in conditions of Yops production. When bacteria carrying the operon fusion were given orally to mice, a clear serum antibody response against CT-B was detected by ELISA. According to immunoblot analysis, this response was only directed against the polymeric form of the B subunit.

L30 ANSWER 69 OF 71 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN DUPLICATE 31
1990:413969 Document No.: PREV199090074770; BA90:74770. EXPRESSION IN ESCHERICHIA-COLI OF TWO MUTATED GENES ENCODING THE **CHOLERA TOXIN** B SUBUNIT. L'HOIR C [Reprint author]; RENARD A; MARTIAL J A. LAB CENTRAL GENIE GENET, B6, UNIV LIEGE, B-4000 SART-TILMAN, BELGIUM. Gene (Amsterdam), (1990) Vol. 89, No. 1, pp. 47-52.
CODEN: GENED6. ISSN: 0378-1119. Language: ENGLISH.

AB To allow subsequent genetically mediated fusion of foreign antigens to **cholera toxin** B subunit (CTB), two mutated CTB encoding genes (**ctxB**) wee constructed and overexpressed in Escherichia coli. The signal peptide coding sequence was deleted and restriction sites were created at both ends of the modified sequence. Both synthesized CTBs contain additional amino acid(s) at the N terminus (one and three). They were purified as insoluble products and refolded into the natural pentameric CTB structure by a denaturation-renaturation cycle. After renaturation, both recombinant proteins recovered CTB antigenicity and the ability to bind to GM1 gangliosides, as shown by in vitro analysis. Preliminary data indicated that both properties were unaltered by fusion of a foreign peptide to the mutated CTBs.

L30 ANSWER 70 OF 71 MEDLINE on STN DUPLICATE 32
90060824. PubMed ID: 2531107. Plasmid vectors for constructing translational fusions to the B subunit of **cholera toxin** . Dertzbaugh M T; Macrina F L. (Department of Microbiology and Immunology, Virginia Commonwealth University, Richmond 23298-0678.) Gene, (1989 Oct 30) Vol. 82, No. 2, pp. 335-42. Journal code: 7706761. ISSN: 0378-1119. Pub. country: Netherlands. Language: English.

AB A family of plasmid cloning vectors has been developed for creating translational fusions to the **ctxB** gene encoding the B subunit of **cholera toxin** (CTB) in Escherichia coli. These vectors permit insertion of transcriptionally and translationally competent gene

sequences upstream from **ctxB**. To test the utility of the system, a portion of the glucosyltransferase B (GTF) gene (gtfB) from the cariogenic bacterium Streptococcus mutans GS-5 (Bratthall serotype c), encoding the N-terminal one-third of the protein, was inserted into each vector. E. coli lysates containing the constructs were partially purified by passage over a GM1 ganglioside affinity column. Western blotting analysis of the column retentate from one of the lysates revealed the presence of a novel 58-kDa protein which cross-reacted with antisera to GTF and CTB. These vectors are of general use for making other translational fusions to **ctxB**. The high binding affinity of CTB can be exploited in purifying large polypeptides fused to this relatively small protein. Moreover, these vectors can be used to create neoantigens with altered immunogenicity for use in polypeptide-based **vaccines**
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L30 ANSWER 71 OF 71 CAPLUS COPYRIGHT 2006 ACS on STN
1984:144966 Document No. 100:144966 Preparation of DNA sequences and recombinant DNA molecules coding for the **cholera toxin** subunits A and B and compositions containing the subunit(s) obtained. Harford, Nigel; De Wilde, Michel (Smith Kline-Rit S. A., Belg.). Eur. Pat. Appl. EP 95452 A2 19831130, 46 pp. DESIGNATED STATES: R: AT, BE, CH, DE, FR, GB, IT, LI, LU, NL, SE. (French). CODEN: EPXXDW. APPLICATION: EP 1983-870048 19830518. PRIORITY: US 1982-381083 19820524.
AB DNA sequences and recombinant DNA mols. containing a region coding for all or part of subunits A and (or) B of **cholera toxin** are prepared from DNA of Vibrio cholerae. **Cholera toxin** subunits are formed in microorganisms transformed with recombinant plasmids. The preparation of a **vaccine** effective against V. cholerae is described. Thus, DNA was isolated from V. cholerae eltor INABA (ATCC 39050) and cleared with ClaI. DNA fragments encoding the A and B subunits of **cholera toxin** (ctxA and **ctxB**, resp.) were identified by Southern hybridizations with genes eltA and eltB, which encode the thermolabile enterotoxin of Escherichia coli. Genes ctxA and **ctxB** were cloned in plasmid pBR322 in E. coli to yield the recombinant plasmids pRIT10841 and pRIT10810, resp., in the E. coli strains ATCC 39053 and ATCC 39051, resp. Plasmid pRIT10814, which contained ctxA and **ctxB** was constructed by the insertion of a ClaI-BglII fragment of pRIT10810 into plasmid pBR327 cleared with ClaI and BamHI to yield pRIT10812. The last plasmid was cleared with ClaI and a ClaI fragment of pRIT10841 was inserted to yield pRIT0814, which was present in E. coli strain ATCC 39052. Exts. of ATCC 39052 gave pos. ileal-loop tests (indicating liquid accumulation) in 9 of 10 rabbits injected. Purified **cholera toxin** (100 ng) gave a pos. response in 8 of 8 rabbits tested; 10 ng of purified toxin gave a pos. response in 3 of 8 rabbits injected. Thus, genes ctxA and **ctxB**, which were joined by their common ClaI site on pRIT10814, yielded a functional determinant for cholera holotoxin. A PstI fragment that contained the 2 genes ctxA and **ctxB** was cloned (in pBR322) directly from DNA of V. cholerae ATCC 39050 to yield the plasmid pRIT10824. No difference in restriction sites in and around the 2 ctx genes was observed between plasmids pRIT10824 and pRIT10841. The sequences of ctxA and **ctxB** genes were determined, and the preparation of an oral **vaccine** with **cholera toxin** subunit B produced in E. coli ATCC 39051 was described.

=> s e coli heat labile enterotoxin
L31 460 E COLI HEAT LABILE ENTEROTOXIN

=> s l31 and Etx
L32 7 L31 AND ETX

=> dup remove l32

PROCESSING COMPLETED FOR L32
L33 3 DUP REMOVE L32 (4 DUPLICATES REMOVED)

=> d l33 1-3 cbib abs

L33 ANSWER 1 OF 3 SCISEARCH COPYRIGHT (c) 2006 The Thomson Corporation on STN
2002:262763 The Genuine Article (R) Number: 529YB. Contribution of the ADP-ribosylating and receptor-binding properties of cholera-like enterotoxins in modulating cytokine secretion by human intestinal epithelial cells. Soriani M; Bailey L; Hirst T R (Reprint). Univ Bristol, Dept Pathol & Microbiol, Bristol BS8 1TD, Avon, England (Reprint). MICROBIOLOGY-SGM (MAR 2002) Vol. 148, Part 3, pp. 667-676. ISSN: 1350-0872 . Publisher: SOC GENERAL MICROBIOLOGY, MARLBOROUGH HOUSE, BASINGSTOKE RD, SPENCERS WOODS, READING RG7 1AG, BERKS, ENGLAND. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS
AB When epithelial cells first encounter cholera toxin (Ctx) produced by Vibrio cholerae they secrete not only chloride ions responsible for causing diarrhoea, but also a number of cytokines that may contribute to the toxin's potent immunomodulatory properties. Much less is known about the ability of the heat-labile enterotoxin of Escherichia coli (**Etx**), a close homologue of Ctx, to elicit cytokine secretion by epithelial cells. This study shows that treatment of human intestinal epithelial T84 cells with **Etx** induces expression of IL-6, IL-10, IL-1R antagonist, as well as IL-1alpha and IL-1beta and low levels of IL-8. Such induction was totally dependent on the intrinsic ADP-ribosylating activity of the toxin A-subunit, and could be mimicked by cAMP-elevating agents, such as forskolin and dibutyryl cAMP. By comparison, neither an enzymically inactive mutant of **Etx** nor EtxB was able to induce cytokine secretion. The behaviour of Ctx and CtxB was very similar to that of **Etx** and EtxB, respectively. The spectrum of cytokines released by **Etx** and Ctx indicates that the toxins may create a local microenvironment that strongly biases the immune response towards an anti-inflammatory and a polarized Th2 response.

L33 ANSWER 2 OF 3 MEDLINE on STN DUPLICATE 1
1999134317. PubMed ID: 9933586. Structural basis for the differential toxicity of cholera toxin and Escherichia coli heat-labile enterotoxin. Construction of hybrid toxins identifies the A2-domain as the determinant of differential toxicity. Rodighiero C; Aman A T; Kenny M J; Moss J; Lencer W I; Hirst T R. (Department of Pathology and Microbiology, University of Bristol, School of Medical Sciences, Bristol BS8 1TD, United Kingdom.) The Journal of biological chemistry, (1999 Feb 12) Vol. 274, No. 7, pp. 3962-9. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.
AB Cholera toxin (Ctx) and **E. coli heat-labile enterotoxin** (**Etx**) are structurally and functionally similar AB5 toxins with over 80% sequence identity. When their action in polarized human epithelial (T84) cells was monitored by measuring toxin-induced Cl- ion secretion, Ctx was found to be the more potent of the two toxins. Here, we examine the structural basis for this difference in toxicity by engineering a set of mutant and hybrid toxins and testing their activity in T84 cells. This revealed that the differential toxicity of Ctx and **Etx** was (i) not due to differences in the A-subunit's C-terminal KDEL targeting motif (which is RDEL in **Etx**), as a KDEL to RDEL substitution had no effect on cholera toxin activity; (ii) not attributable to the enzymatically active A1-fragment, as hybrid toxins in which the A1-fragment in Ctx was substituted for that of **Etx** (and vice versa) did not alter relative toxicity; and (iii) not due to the B-subunit, as the replacement of the B-subunit in Ctx for that of **Etx** caused no alteration in toxicity, thus excluding the possibility that the broader receptor specificity of EtxB is responsible for reduced activity. Remarkably, the

difference in toxicity could be mapped to a 10-amino acid segment of the A2-fragment that penetrates the central pore of the B-subunit pentamer. A comparison of the in vitro stability of two hybrid toxins, differing only in this 10-amino acid segment, revealed that the Ctx A2-segment conferred a greater stability to the interaction between the A- and B-subunits than the corresponding segment from **Etx** A2. This suggests that the reason for the relative potency of Ctx compared with **Etx** stems from the increased ability of the A2-fragment of Ctx to maintain holotoxin stability during uptake and transport into intestinal epithelia.

L33 ANSWER 3 OF 3 CAPLUS COPYRIGHT 2006 ACS on STN
1992:528012 Document No. 117:128012 A homolog of the Escherichia coli DsbA protein involved in disulfide bond formation is required for enterotoxin biogenesis in Vibrio cholerae. Yu, Jun; Webb, Helen; Hirst, Timothy R. (Biol. Lab., Univ. Kent, Canterbury/Kent, CT2 7NJ, UK). Molecular Microbiology, 6(14), 1949-58 (English) 1992. CODEN: MOMIEE. ISSN: 0950-382X.

AB A strain of V. cholerae, which had been engineered to express high levels of the non-toxic B subunit (EtxB) of **E. coli heat-labile enterotoxin**, was subjected to transposon (TnphoA) mutagenesis. Two chromosomal TnphoA insertion mutations of the strain were isolated that showed a severe defect in the amount of EtxB produced. The loci disrupted by TnphoA in the two mutant derivs. were cloned and sequenced, and this revealed that the transposon had inserted at different sites in the same gene. The open reading frame of the gene predicts a 200-amino-acid exported protein, with a Cys-X-X-Cys motif characteristic of thioredoxin, protein disulfide isomerase, and DsbA (a periplasmic protein required for disulfide bond formation in E. coli). The V. cholerae protein exhibited 40% identity with the DsbA protein of E. coli, including 90% identity in the region of the active-site motif. Introduction of a plasmid encoding E. coli DsbA into the V. cholerae TnphoA derivs. was found to restore enterotoxin formation, while expression of **Etx** or EtxB in a dsbA mutant of E. coli confirmed that DsbA is required for enterotoxin formation in E. coli. These results suggest that, since each EtxB subunit contains a single intramol. disulfide bond, a transient intermol. interaction with DsbA occurs during toxin subunit folding which catalyzes formation of the disulfide in vivo.

=> s l31 and "EtxB"
L34 36 L31 AND "ETXB"

=> dup remove l34
PROCESSING COMPLETED FOR L34
L35 13 DUP REMOVE L34 (23 DUPLICATES REMOVED)

=> d l35 1-13 cbib abs

L35 ANSWER 1 OF 13 MEDLINE on STN DUPLICATE 1
2004311350. PubMed ID: 15213152. Nasal delivery of antigen with the B subunit of Escherichia coli heat-labile enterotoxin augments antigen-specific T-cell clonal expansion and differentiation. Apostolaki Maria; Williams Neil A. (Department of Pathology and Microbiology, School of Medical Sciences, University of Bristol, University Walk, Bristol, United Kingdom.) Infection and immunity, (2004 Jul) Vol. 72, No. 7, pp. 4072-80. Journal code: 0246127. ISSN: 0019-9567. Pub. country: United States. Language: English.

AB Escherichia coli heat-labile enterotoxin has unique immunogenic and adjuvant properties when administered mucosally to mice. These properties have revealed the potential for its use in the development of mucosal vaccines, an area of increasing interest. However, the inherent toxicity mediated by the A subunit precludes its widespread use. This problem has led to attempts to dissociate toxicity from adjuvant function by use of

the B subunit. The ability of the B subunit of **E. coli heat-labile enterotoxin** (**EtxB**) to enhance responses against antigens coadministered intranasally is demonstrated here with the use of the DO11.10 adoptive-transfer model, in which ovalbumin (OVA)-specific adoptively transferred T cells can be monitored directly by flow cytometry. Intranasal delivery of OVA with **EtxB** resulted in increased T-cell proliferative and systemic antibody responses against antigens. The increased Th2 cytokine production detected following in vitro restimulation of splenocyte and cervical lymph node (CLN) cells from the immunized mice correlated with increased OVA-specific immunoglobulin G1 antibody production. Flow cytometric analysis of T cells from mice early after immunization directly revealed the ability of **EtxB** to support antigen-specific clonal expansion and differentiation. Furthermore, while responses were first detected in the CLNs, they rapidly progressed to the spleen, where they were further sustained. Examination of CD69 expression on dividing cells supported the notion that activation induced by the presence of antigens is not sufficient to drive T-cell differentiation. Furthermore, a lack of CD25 expression on dividing cells suggested that **EtxB**-mediated T-cell clonal expansion may occur without a sustained requirement for interleukin 2.

L35 ANSWER 2 OF 13 SCISEARCH COPYRIGHT (c) 2006 The Thomson Corporation on STN

2002:262763 The Genuine Article (R) Number: 529YB. Contribution of the ADP-ribosylating and receptor-binding properties of cholera-like enterotoxins in modulating cytokine secretion by human intestinal epithelial cells. Soriani M; Bailey L; Hirst T R (Reprint). Univ Bristol, Dept Pathol & Microbiol, Bristol BS8 1TD, Avon, England (Reprint). MICROBIOLOGY-SGM (MAR 2002) Vol. 148, Part 3, pp. 667-676. ISSN: 1350-0872 . Publisher: SOC GENERAL MICROBIOLOGY, MARLBOROUGH HOUSE, BASINGSTOKE RD, SPENCERS WOODS, READING RG7 1AG, BERKS, ENGLAND. Language: English. *ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS*

AB When epithelial cells first encounter cholera toxin (Ctx) produced by Vibrio cholerae they secrete not only chloride ions responsible for causing diarrhoea, but also a number of cytokines that may contribute to the toxin's potent immunomodulatory properties. Much less is known about the ability of the heat-labile enterotoxin of Escherichia coli (Etx), a close homologue of Ctx, to elicit cytokine secretion by epithelial cells. This study shows that treatment of human intestinal epithelial T84 cells with Etx induces expression of IL-6, IL-10, IL-1R antagonist, as well as IL-1alpha and IL-1beta and low levels of IL-8. Such induction was totally dependent on the intrinsic ADP-ribosylating activity of the toxin A-subunit, and could be mimicked by cAMP-elevating agents, such as forskolin and dibutyryl cAMP. By comparison, neither an enzymically inactive mutant of Etx nor **EtxB** was able to induce cytokine secretion. The behaviour of Ctx and CtxB was very similar to that of Etx and **EtxB**, respectively. The spectrum of cytokines released by Etx and Ctx indicates that the toxins may create a local microenvironment that strongly biases the immune response towards an anti-inflammatory and a polarized Th2 response.

L35 ANSWER 3 OF 13 MEDLINE on STN DUPLICATE 2

1999134317. PubMed ID: 9933586. Structural basis for the differential toxicity of cholera toxin and Escherichia coli heat-labile enterotoxin. Construction of hybrid toxins identifies the A2-domain as the determinant of differential toxicity. Rodighiero C; Aman A T; Kenny M J; Moss J; Lencer W I; Hirst T R. (Department of Pathology and Microbiology, University of Bristol, School of Medical Sciences, Bristol BS8 1TD, United Kingdom.) The Journal of biological chemistry, (1999 Feb 12) Vol. 274, No. 7, pp. 3962-9. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB Cholera toxin (Ctx) and **E. coli heat-**

labile enterotoxin (Etx) are structurally and functionally similar AB5 toxins with over 80% sequence identity. When their action in polarized human epithelial (T84) cells was monitored by measuring toxin-induced Cl- ion secretion, Ctx was found to be the more potent of the two toxins. Here, we examine the structural basis for this difference in toxicity by engineering a set of mutant and hybrid toxins and testing their activity in T84 cells. This revealed that the differential toxicity of Ctx and Etx was (i) not due to differences in the A-subunit's C-terminal KDEL targeting motif (which is RDEL in Etx), as a KDEL to RDEL substitution had no effect on cholera toxin activity; (ii) not attributable to the enzymatically active A1-fragment, as hybrid toxins in which the A1-fragment in Ctx was substituted for that of Etx (and vice versa) did not alter relative toxicity; and (iii) not due to the B-subunit, as the replacement of the B-subunit in Ctx for that of Etx caused no alteration in toxicity, thus excluding the possibility that the broader receptor specificity of **EtxB** is responsible for reduced activity. Remarkably, the difference in toxicity could be mapped to a 10-amino acid segment of the A2-fragment that penetrates the central pore of the B-subunit pentamer. A comparison of the in vitro stability of two hybrid toxins, differing only in this 10-amino acid segment, revealed that the Ctx A2-segment conferred a greater stability to the interaction between the A- and B-subunits than the corresponding segment from Etx A2. This suggests that the reason for the relative potency of Ctx compared with Etx stems from the increased ability of the A2-fragment of Ctx to maintain holotoxin stability during uptake and transport into intestinal epithelia.

L35 ANSWER 4 OF 13 MEDLINE on STN DUPLICATE 3
1999171729. PubMed ID: 10073721. Immunogenicity of Actinobacillus ApxIA toxin epitopes fused to the **E. coli heat-labile enterotoxin** B subunit. Bagdasarian M M; Nagai M; Frey J; Bagdasarian M. (Department of Microbiology, Michigan State University, East Lansing 48824-1312, USA.) Vaccine, (1999 Feb 5) Vol. 17, No. 5, pp. 441-7. Journal code: 8406899. ISSN: 0264-410X. Pub. country: ENGLAND: United Kingdom. Language: English.

AB Peptides KDYGASTGSSL (Epi1). SLLRRRRNGEDVSV (Epi3) and DDEIYGNDGHP (Epi6), predicted to constitute immunogenic epitopes of the hemolysin-cytotoxin ApxIA of Actinobacillus pleuropneumoniae were inserted into a surface-exposed loop of the B subunit of the **E. coli heat-labile enterotoxin** (**EtxB**). The resulting chimeric proteins were recognized by monospecific antibodies against purified native ApxI and by convalescent sera of pigs that were positive for A. pleuropneumoniae serotype 1. Mice anti-sera against chimeric proteins **EtxB**::ApxIAEpi3 and **EtxB**::ApxIAEpi6 reacted with purified ApxI. These results indicate that Epi3 and Epi6 regions constitute linear epitopes of the structural ApxIA protein toxin. Epitope Epi6 which is located in the structure of the glycine rich repeats in ApxI elicits the formation of hemolysin neutralizing antibodies when introduced into mice in the form of a chimeric **EtxB** fusion protein. We suggest that fusion of peptide sequences to **EtxB** is a useful tool for the analysis of epitopes of complex proteins such as RTX toxins.

L35 ANSWER 5 OF 13 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN
2000:155508 Document No.: PREV200000155508. Signalling events induced by **E. coli heat-labile enterotoxin** B-subunit-receptor binding. Bone, Heather K. [Reprint author]; Pitman, Richard S. [Reprint author]; Williams, Neil A. [Reprint author]. Dept. Pathology and Microbiology, University of Bristol, Bristol, BS8 1TD, UK. Immunology, (Dec., 1999) Vol. 98, No. suppl. 1, pp. 174. print.
Meeting Info.: Joint Congress of the British Society for Immunology and the British Society for Allergy and Clinical Immunology. Harrogate,

England, UK. November 30-December 03, 1999. British Society for Allergy and Clinical Immunology; British Society for Immunology. CODEN: IMMUAM. ISSN: 0019-2805. Language: English.

L35 ANSWER 6 OF 13 MEDLINE on STN DUPLICATE 4
1998379563. PubMed ID: 9713936. Display of an inhibin epitope in a surface-exposed loop of the **E. coli heat-labile enterotoxin** B subunit. Sewani C R; Bagdasarian M M; Ireland J J; Bagdasarian M. (Department of Physiology, Michigan State University, East Lansing 48824-1312, USA.) Vaccine, (1998 Oct) Vol. 16, No. 17, pp. 1611-9. Journal code: 8406899. ISSN: 0264-410X. Pub. country: ENGLAND: United Kingdom. Language: English.

AB In vitro gene manipulation was used to develop a novel chimeric antigen consisting of the non-toxic B subunit (**EtxB**) of an E. coli enterotoxin and the first 14 N-terminal amino acid residues of the carboxy-terminal portion of the alpha subunit of bovine inhibin (bINH1-14). Rabbits immunized subcutaneously (s.c.) or intravenously (i.v.) with **EtxB**::bINH1-14, with or without Freund's adjuvant, developed significant titres of antibodies that recognized an inhibin peptide fragment containing bINH1-14, native inhibins, and **EtxB** during separate enzyme-linked immunosorbent assay (ELISA). Passive immunization of mice with the rabbit anti-**EtxB**::bINH1-14 serum increased concentrations of follicle-stimulating hormone (FSH) in serum twofold compared with controls, whereas serum concentrations of luteinizing hormone (LH) were unaltered. Since FSH is the primary hormone from the pituitary gland that stimulates ovarian follicle growth and spermatogenesis, the results of this study demonstrate that **EtxB** ::bINH1-14 has potential as antigen for development of inhibin-based fertility vaccines.

L35 ANSWER 7 OF 13 EMBASE COPYRIGHT (c) 2006 Elsevier B.V. All rights reserved on STN DUPLICATE 5
97248420 EMBASE Document No.: 1997248420. EDTA protects **E. coli heat-labile enterotoxin** B subunit-based fusion proteins from proteolytic degradation during their production by Vibrio spp. Loregian A.; Marcello A.; Hirst T.R.; Palu G.. G. Palu, Institute of Microbiology, University of Padova, Via A. Gabelli 63, 35121 Padova, Italy. Minerva Biotecnologica Vol. 9, No. 2, pp. 61-67 1997.
Refs: 35.
ISSN: 1120-4826. CODEN: MIBIFK
Pub. Country: Italy. Language: English. Summary Language: English.
Entered STN: 970904. Last Updated on STN: 970904

AB **E. coli heat-labile enterotoxin** B subunit (**EtxB**) has been proposed as a potential protein carrier for the delivery of heterologous peptides into target cells. To thoroughly exploit the biotechnological potential of **EtxB**- based fusion proteins, a simple method has to be worked out for their expression and purification. Production of these chimeric toxins faces problems with regard to both their low yield and stability. Methods. In this study we describe a protocol for the optimal production and secretion of undegraded **EtxB** hybrids into the extracellular medium of cultures of non- toxinogenic Vibrio strains. Results. The highest level of expression of two chimeric toxins, **EtxB**-R2 and **EtxB**-pol, was obtained by using Vibrio sp. 60 cultures, reaching 52 mg/l and 8 mg/l, respectively. The presence of 0.3 mM EDTA in the culture medium totally preserved both chimeric toxins from proteolysis, also during a prolonged expression. Conclusions. This system may be useful for the preparation of other **EtxB**-based fusion proteins.

L35 ANSWER 8 OF 13 CAPLUS COPYRIGHT 2006 ACS on STN
1996:37824 Document No. 124:84293 Potent immunogenicity of the B subunits of Escherichia coli heat-labile enterotoxin: receptor binding is essential

and induces differential modulation of lymphocyte subsets. Nashar, Toufic O.; Webb, Helen M.; Eaglestone, Simon; Williams, Neil A.; Hirst, Timothy R. (Res. School Biosciences, Univ. Kent, Kent, CT2 7NJ, UK). Proceedings of the National Academy of Sciences of the United States of America, 93(1), 226-30 (English) 1996. CODEN: PNASA6. ISSN: 0027-8424. Publisher: National Academy of Sciences.

AB The importance of receptor binding in the potent immunogenicity of **E. coli heat-labile enterotoxin** B subunit (**EtxB**) was tested by comparing its immunol. properties with those of a receptor binding mutant, **EtxB** (G33D). S.c. immunization of **EtxB**(G33D) resulted in 160-fold reduction in antibody titer compared with wild-type **EtxB**, whereas its oral delivery failed to provoke any detectable secretory or serum anti-B subunit responses. Moreover, the 2 proteins induced strikingly different effects on lymphocyte cultures in vitro. **EtxB**, in comparison with **EtxB**(G33D), caused an increase in the proportion of B cells, many of which were activated (CD25+); the complete depletion of CD8+ T cells; an increase in the activation of CD4+ T cells; and an increase in interleukin 2 and a decrease in interferon γ. Thus, **EtxB** exerts profound effects on immune cells, suggesting that its potent immunogenicity is dependent not only on efficient receptor-mediated uptake, but also on direct receptor-mediated immunomodulation of lymphocyte subsets.

L35 ANSWER 9 OF 13 MEDLINE on STN DUPLICATE 6

95378272. PubMed ID: 7544352. Generation of a monoclonal antibody that recognizes the amino-terminal decapeptide of the B-subunit of Escherichia coli heat-labile enterotoxin. A new probe for studying toxin assembly intermediates. Amin T; Larkins A; James R F; Hirst T R. (Research School of Biosciences, University of Kent, Canterbury, United Kingdom.) The Journal of biological chemistry, (1995 Aug 25) Vol. 270, No. 34, pp. 20143-50. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB Cholera toxin and the related Escherichia coli heat-labile enterotoxin are hexameric proteins comprising one A-subunit and five B-subunits. In this paper we report the generation and characterization of a monoclonal antibody, designated LDS47, that recognizes and precipitates in vivo assembly intermediates of the B-subunit (**EtxB**) of **E. coli heat-labile enterotoxin.** The monoclonal antibody is unable to precipitate native B-subunit pentamers, thus making LDS47 a useful probe for studying the early stages of enterotoxin biogenesis. The use of LDS47 to monitor the in vivo turnover of newly synthesized B-subunits in the periplasm of E. coli demonstrated that (i) the turnover of unassembled B-subunits followed an apparent first order process and (ii) it occurred concomitantly with the assembly of native B-pentamers (k = 0.317 +/- 0.170 min-1; t1/2 = 2.2 min). No other proteins were co-precipitated with the newly synthesized B-subunits; a finding that implies that unassembled B-subunits do not stably associate with other periplasmic proteins prior to their assembly into a macromolecular complex. The use of overlapping synthetic peptides corresponding to the entire **EtxB** polypeptide demonstrated that the epitope recognized by LDS47 is located within the amino-terminal decapeptide of the B-subunit. From the x-ray structural analysis of the toxin (Sixma, T., Kalk, K., van Zanten, B., Dauter, Z., Kingma, J., Witholt, B., and Hol, W. G. J. (1993) J. Mol. Biol. 230, 890-918), this region appears to resemble a curved finger that clasps the adjacent B-subunit. Thus, this region might be expected to be exposed in the unfolded or unassembled subunit, but to become partially buried upon assembly and thus inaccessible to recognition by the monoclonal antibody.

L35 ANSWER 10 OF 13 CAPLUS COPYRIGHT 2006 ACS on STN

1995:404765 Document No. 122:233725 Construction of a fusion protein between B subunit of **E. coli heat-labile**

enterotoxin and the C-terminus of herpes simplex virus-DNA polymerase. Loregian, Arianna; Marcello, Alessandro; Hirst, Timothy R.; Marsden, Howard S.; Palu, Giorgio (Institute of Microbiology, Univ. of Padova, Italy). Biochemical Society Transactions, 23(1), 61S (English) 1995. CODEN: BCSTB5. ISSN: 0300-5127. Publisher: Portland Press.

AB It was recently reported that the B subunit of heat-labile enterotoxin from Escherichia coli (**EtxB**) could be used as a recombinant carrier for the receptor-mediated delivery of a peptide fused to it. This was further examined here by characterizing the fusion protein obtained by genetically linking the C-terminal 27 amino acids of HSV-1 DNA polymerase to the C-terminus of **EtxB** (**EtxB**-DNApol). The novel polypeptide was overexpressed in E. coli XL1-Blue and shown to be translocated to the periplasmic compartment at an approx. 10-fold lower level than wild-type **EtxB** expressed under the same conditions. The same experiment also indicated that **EtxB**-DNApol was properly assembled into pentamers capable of binding GM1.

L35 ANSWER 11 OF 13 MEDLINE on STN DUPLICATE 7

94237439. PubMed ID: 8181710. Efficient extracellular production of hybrid **E. coli heat-labile enterotoxin** B subunits in a marine Vibrio. Marcello A; Loregian A; Palu G; Hirst T R. (Institute of Microbiology, University of Padua, Italy.) FEMS microbiology letters, (1994 Mar 15) Vol. 117, No. 1, pp. 47-51. Journal code: 7705721. ISSN: 0378-1097. Pub. country: Netherlands. Language: English.

AB Escherichia coli heat-labile enterotoxin B subunit (**EtxB**) has been proposed as a potential protein carrier for the delivery of heterologous peptides to target cells, particularly for the oral delivery of epitopes to the mucosal immune system. In this study, two extensions to the C-terminus of **EtxB** were genetically engineered that correspond, to a well-characterized neutralising epitope of glycoprotein D from herpes simplex virus (**EtxB**-gD) and to the C-terminal nine amino acids from the 38 kDa subunit of HSV-encoded ribonucleotide reductase (**EtxB**-R2). Here we describe the extracellular secretion of the two hybrid **EtxBs** from a marine Vibrio harbouring a broad-host range inducible expression vector containing the hybrid genes. Large amounts of intact fusion proteins (15-20 mg per liter of culture) were secreted into the medium upon induction. These hybrid proteins maintained the receptor-binding activity of the native toxin as well as being cross-reactive with anti-**EtxB** and anti-heterologous peptide monoclonal antibodies.

L35 ANSWER 12 OF 13 CAPLUS COPYRIGHT 2006 ACS on STN

1992:528012 Document No. 117:128012 A homolog of the Escherichia coli DsbA protein involved in disulfide bond formation is required for enterotoxin biogenesis in Vibrio cholerae. Yu, Jun; Webb, Helen; Hirst, Timothy R. (Biol. Lab., Univ. Kent, Canterbury/Kent, CT2 7NJ, UK). Molecular Microbiology, 6(14), 1949-58 (English) 1992. CODEN: MOMIEE. ISSN: 0950-382X.

AB A strain of V. cholerae, which had been engineered to express high levels of the non-toxic B subunit (**EtxB**) of **E. coli heat-labile enterotoxin**, was subjected to transposon (TnphoA) mutagenesis. Two chromosomal TnphoA insertion mutations of the strain were isolated that showed a severe defect in the amount of **EtxB** produced. The loci disrupted by TnphoA in the two mutant derivs. were cloned and sequenced, and this revealed that the transposon had inserted at different sites in the same gene. The open reading frame of the gene predicts a 200-amino-acid exported protein, with a Cys-X-X-Cys motif characteristic of thioredoxin, protein disulfide isomerase, and DsbA (a periplasmic protein required for disulfide bond formation in E. coli). The V. cholerae protein exhibited 40% identity with the DsbA protein of E. coli, including 90% identity in the region of the active-site motif. Introduction of a plasmid encoding E. coli DsbA

into the V. cholerae TnphoA derivs. was found to restore enterotoxin formation, while expression of Etx or **EtxB** in a dsbA mutant of E. coli confirmed that DsbA is required for enterotoxin formation in E. coli. These results suggest that, since each **EtxB** subunit contains a single intramol. disulfide bond, a transient intermol. interaction with DsbA occurs during toxin subunit folding which catalyzes formation of the disulfide in vivo.

L35 ANSWER 13 OF 13 CAPLUS COPYRIGHT 2006 ACS on STN
1992:402474 Document No. 117:2474 Expression of the B subunit of Escherichia coli heat-labile enterotoxin in a marine Vibrio and in a mutant that is pleiotropically defective in the secretion of extracellular proteins. Leece, Robin; Hirst, Timothy R. (Dep. Genet., Univ. Leicester, Leicester, LE1 7RH, UK). Journal of General Microbiology, 138(4), 719-24 (English) 1992. CODEN: JGMIAN. ISSN: 0022-1287.

AB A marine Vibrio (designated Vibrio sp. 60) that is related to V. anguillarum was used as a host for a plasmid that encodes the non-toxic B subunit (**EtxB**) of **E. coli heat-labile enterotoxin**. Expression of **EtxB** in Vibrio sp. 60 resulted in the efficient and selective secretion of the B subunit into the extracellular growth medium. This indicated that Vibrio sp. 60, which does not normally produce cholera-like enterotoxins, nonetheless possesses a secretory machinery that permits these toxins to be translocated across its cytoplasmic and outer membranes. Expression of **EtxB** in a sec mutant of Vibrio sp. 60 (MVT1192), which had previously been shown to be defective in the secretion of several extracellular proteins, resulted in approx. 95% of the B subunit remaining entrapped within the periplasm of the bacterial cell envelope. This implies that the mutation in MVT1192 defines a locus that dets. a common step in the secretion of extracellular proteins, including oligomeric toxins.

```
=> s l31 and eczema
L36          0 L31 AND ECZEMA

=> s l31 and dermatitis
L37          0 L31 AND DERMATITIS

=> s l31 and asthma
L38          0 L31 AND ASTHMA

=> s l31 and uticaria
L39          0 L31 AND UTICARIA

=> s l31 and hives
L40          0 L31 AND HIVES

=> s l31 and hypersensitivity
L41          0 L31 AND HYPERSENSITIVITY

=> s (williams n?/au or hirst t?/au or bienenstock j?/au)
L42       9977 (WILLIAMS N?/AU OR HIRST T?/AU OR BIENENSTOCK J?/AU)

=> s l42 and toxin
L43        484 L42 AND TOXIN

=> s l43 and allergic
L44          6 L43 AND ALLERGIC

=> dup remove l44
PROCESSING COMPLETED FOR L44
L45          5 DUP REMOVE L44 (1 DUPLICATE REMOVED)
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=> d l45 1-5 cbib abs

L45 ANSWER 1 OF 5 EMBASE COPYRIGHT (c) 2006 Elsevier B.V. All rights reserved on STN

2004275532 EMBASE Modulation of the immune response by the cholera-like enterotoxins. Plant A.; **Williams N.A.**. N.A. Williams, University of Bristol, Department of Pathology/Microbiology, School of Medical Sciences, University Walk, Bristol BS8 1TD, United Kingdom. neil.a.williams@bris.ac.uk. Current Topics in Medicinal Chemistry Vol. 4, No. 5, pp. 509-519 2004.
Refs: 112.
ISSN: 1568-0266. CODEN: CTMCCL
Pub. Country: Netherlands. Language: English. Summary Language: English.
Entered STN: 20040715. Last Updated on STN: 20040715

AB Cholera **toxins** and heat labile enterotoxin from E. coli differ from most soluble proteins in eliciting systemic immunity both against themselves and unrelated admixed antigens, rather than tolerance following administration to a mucosal surface. Several reports have also demonstrated preferential induction of Th2-type responses when these molecules are used as adjuvants. Conversely, these proteins and their non-toxic derivatives, including the B sub-units are also able prevent and alleviate autoimmune diseases in naive and systemically immune hosts demonstrating wide-ranging effects on the immune system. The recent observation that amelioration of autoimmune disease is associated with the generation of regulatory T cells which inhibit pathogenic Th1 responses may also help to consolidate these two apparently contradictory outcomes of exposure to the cholera-like enterotoxins. Furthermore, the observation that EtxB is able to alleviate autoimmune disease in the absence of conjugation to autoantigen highlights its potential for use in the clinical setting where the target antigen is often unknown. Direct effects on T cells, B cells and APC have been demonstrated in vitro which have provided insights into how these molecules may elicit these diverse effects. Further investigation is required for elucidation of the mechanisms of action of adjuvanticity and tolerance induction by these molecules to realise their potential for use in vaccines and therapies for autoimmune disease in humans. .COPYRGT. 2004 Bentham Science Publishers Ltd.

L45 ANSWER 2 OF 5 EMBASE COPYRIGHT (c) 2006 Elsevier B.V. All rights reserved on STN DUPLICATE 1

2002036163 EMBASE Relationships between mast cells and the nervous system. **Bienenstock J.**. J. Bienenstock, McMaster University, 1200 Main Street West, Hamilton, Ont. L8N 3Z5, Canada. bienens@mcmaster.ca. Revue Francaise d'Allergologie et d'Immunologie Clinique Vol. 42, No. 1, pp. 11-15 2002.
Refs: 47.
ISSN: 0335-7457. CODEN: RFAIBB
Pub. Country: France. Language: English. Summary Language: English.
Entered STN: 20020207. Last Updated on STN: 20020207

AB We and others have shown bi-directional mast cell communication with nerves both in vitro and in vivo. Many examples of this now exist in terms of skin, lung, intestine and urinary bladder both in vitro and in vivo. This communication appears to be important in a variety of situations relating to emotion, behaviour, stress and **allergic** and other hypersensitivity reactions, and these interactions appear to be important also in host resistance and responses to **toxins**. It is reasonable to conclude that mast cells and nerves can form a homeostatic unit, that mast cells are present in part to act as relays of environmental information to the nervous system, and that in turn they can be involved in the regulation of immune and inflammatory responses. They can act as a switchboard in this latter condition, or as a regulatory gateway in control of the release of hormones in the HPA axis. Mast cells

are multifunctional cells and when associated with nerves, these functions are further amplified, and in this sense they can act both as conductors of the orchestra as well as players within it. .COPYRGT. 2002 Editions scientifiques et medicales Elsevier SAS.

L45 ANSWER 3 OF 5 CAPLUS COPYRIGHT 2006 ACS on STN
1999:451202 Document No. 131:82960 EtxB or ganglioside GM1 for treating **allergic** or hypersensitivity conditions. **Williams, Neil Andrew; Hirst, Timothy Raymond; Bienenstock, John** (Oratol Limited, UK). PCT Int. Appl. WO 9934817 A1 19990715, 46 pp. DESIGNATED STATES: W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1999-GB70 19990108. PRIORITY: GB 1998-487 19980109.
AB The use of an agent in the manufacture of a medicament to treat an **allergic** condition and/or a hypersensitivity condition is described. The agent is capable of modulating a ganglioside-associated activity. The agent is not coupled to an antigen. The modulation of the ganglioside-associated activity affects an **allergic** condition and/or a hypersensitivity condition. Examples of such modulators include ganglioside GM1 and E. coli enterotoxin B subunit.

L45 ANSWER 4 OF 5 EMBASE COPYRIGHT (c) 2006 Elsevier B.V. All rights reserved on STN
1999105073 EMBASE Immune modulation by the cholera-like enterotoxins: From adjuvant to therapeutic. **Williams N.A.; Hirst T.R.;** Nashar T.O.. N.A. Williams, University of Bristol, Dept. of Pathology and Microbiology, School of Medical Sciences, Bristol BS8 1TD, United Kingdom. neil.a.william@bris.ac.uk. Immunology Today Vol. 20, No. 2, pp. 95-101 1999.
Refs: 63.
ISSN: 0167-5699. CODEN: IMTOD8
S 0167-5699(98)01397-8. Pub. Country: United Kingdom. Language: English. Summary Language: English.
Entered STN: 19990429. Last Updated on STN: 19990429
AB Cholera **toxin** and its close relative, Escherichia coli heat-labile enterotoxin, are potent immunogens and mucosal adjuvants. The recent findings that their B subunits can promote tolerance highlights the complexity of their interactions with the immune system. Here, Neil Williams and colleagues review the mechanisms by which these molecules and seek to explain the paradox.

L45 ANSWER 5 OF 5 SCISEARCH COPYRIGHT (c) 2006 The Thomson Corporation on STN
1995:2267 The Genuine Article (R) Number: PX275. PERTUSSIS **TOXIN** STIMULATES HYPERSENSITIVITY AND ENHANCES NERVE-MEDIATED ANTIGEN UPTAKE IN RAT INTESTINE. KOSECKA U (Reprint); MARSHALL J S; CROWE S E; **BIENENSTOCK J**; PERDUE M H. MCMASTER UNIV, DEPT PATHOL, INTESTINAL DIS RES PROGRAM, HAMILTON, ON L8N 3Z5, CANADA; MCMASTER UNIV, DEPT PATHOL, MOLEC VIROL & IMMUNOL PROGRAM, HAMILTON, ON L8N 3Z5, CANADA. AMERICAN JOURNAL OF PHYSIOLOGY-GASTROINTESTINAL AND LIVER PHYSIOLOGY (NOV 1994) Vol. 30, No. 5, pp. G745-G753. ISSN: 0193-1857. Publisher: AMER PHYSIOLOGICAL SOC, 9650 ROCKVILLE PIKE, BETHESDA, MD 20814. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS
AB Pertussis **toxin** stimulates hypersensitivity and enhances nerve-mediated antigen uptake in rat intestine. Am. J. Physiol. 267 (Gastrointest. Liver Physiol. 30): G745-G753, 1994.-We previously reported that intestine from rats sensitized to ovalbumin (Oval, using

Bordetella pertussis vaccine as adjuvant, demonstrated a rapid secretory response [increase in short-circuit current (I-sc)]. to Ova upon secondary challenge. Here, we examined the role of pertussis **toxin**, the active component of the vaccine, in the response. Sensitization of Sprague-Dawley rats by intraperitoneal injection of recombinant wild-type pertussis **toxin** (wPT) plus Ova enhanced intestinal responses (at day 14: similar to 20-fold for luminal antigen, similar to 2.5-fold for serosal antigen) compared with rats sensitized by injection of Ova alone. In contrast, sensitization with an enzymatically inactive mutant pertussis **toxin** (wPT, different in two amino acids) produced no significant effect. Ova-specific immunoglobulin (Ig) E and IgG(2a) antibodies and greater numbers of mucosal mast cells were documented in wPT-sensitized rats. In addition, the I-sc response to electrical transmural stimulation of nerves in intestinal preparations was significantly augmented. Neurotoxin inhibited the secretory response to luminal but not serosal antigen. Immunophysiological stimulation by wPT was still evident 8 mo postsensitization. Our studies indicate that pertussis **toxin** causes longlasting hypersensitivity to coadministered antigens, involving increased production of reaginic antibodies, hyperplasia of mucosal mast cells, and enhanced neurally mediated uptake of antigen across the intestinal epithelium. These findings suggest a potential role for bacterial products in the development of immunophysiological reactions to ingested antigens.

```
=> s l43 and cholera toxin
L46         274 L43 AND CHOLERA TOXIN

=> s l46 and enterotoxin
L47         212 L46 AND ENTEROTOXIN

=> s l47 and treatment
L48          18 L47 AND TREATMENT

=> dup reomve l48
ENTER REMOVE, IDENTIFY, ONLY, OR (?):remove
'REOMVE' IS NOT VALID.  VALID FILE NAMES ARE 'MEDLINE, EMBASE, BIOSIS, SCISEARCH,
CAPLUS'
You have entered a file name of duplicates to keep that is not
referenced by any of the L#s specified for this DUPLICATE command.
The file names of duplicates that can be kept are listed above.
Please enter one of these file names.

=> dup remove l48
PROCESSING COMPLETED FOR L48
L49          11 DUP REMOVE L48 (7 DUPLICATES REMOVED)

=> d l49 1-11 cbib abs

L49  ANSWER 1 OF 11  SCISEARCH  COPYRIGHT (c) 2006 The Thomson Corporation  on
     STN
2006:77280   The Genuine Article (R) Number: 003GB. Protection of non-obese
     diabetic mice from autoimmune diabetes by Escherichia coli heat-labile
     enterotoxin B subunit.  Ola T O; Williams N A (Reprint).
     Univ Bristol, Sch Med Sci, Dept Cellular & Mol Med, Univ Walk, Bristol BS8
     1TD, Avon, England (Reprint); Univ Bristol, Sch Med Sci, Dept Pathol &
     Microbiol, Bristol BS8 1TD, Avon, England. t.o.ola@qmul.ac.uk;
     neil.a.williams@bristol.ac.uk. IMMUNOLOGY (FEB 2006) Vol. 117, No. 2, pp.
     262-270. ISSN: 0019-2805. Publisher: BLACKWELL PUBLISHING, 9600 GARSINGTON
     RD, OXFORD OX4 2DQ, OXON, ENGLAND. Language: English.
     *ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS*
AB        Autoimmune diabetes in the non-obese diabetic (NOD) mouse is
     associated with development of inflammation around the islets at around
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4-5 weeks of age, which may be prolonged until frank diabetes begins to occur around 12 weeks of age. Although many interventions can halt disease progression if administration coincides with the beginning of the anti-beta cell response, very few are able to prevent diabetes development once insulitis is established. Here we describe a strategy which blocks cellular infiltration of islets and prevents diabetes. Intranasal **treatment** with the B-subunit of Escherichia coli heat labile **enterotoxin** (EtxB), a protein that binds GM1 ganglioside (as well as GD1b, asialo-GM1 and lactosylceramide with lower affinities), protected NOD mice from developing diabetes in a receptor-binding dependent manner. Protection was associated with a significant reduction in the number of macrophages, CD4(+) T cells, B cells, major histocompatibility complex class II+ cells infiltrating the islets. Despite this, treated mice showed increased number of interleukin-10(+) cells in the pancreas, and a decrease in both T helper 1 (Th1) and Th2 cytokine production in the pancreatic lymph node. Disease protection was also transferred with CD4(+) splenocytes from treated mice. Taken together, these results demonstrated that EtxB is a potent immune modulator capable of blocking diabetes.

L49 ANSWER 2 OF 11 CAPLUS COPYRIGHT 2006 ACS on STN

2003:6139 Document No. 138:68275 Mutant forms of **enterotoxin** (EtxB) and **cholera toxin** (CtxB), and their therapeutic uses as target site-specific carriers. **Hirst, Timothy Raymond** (University of Bristol, UK). PCT Int. Appl. WO 2003000899 A1 20030103, 84 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2002-GB2829 20020620. PRIORITY: GB 2001-15382 20010622.

AB The present invention describes the use of a mutant form of **enterotoxin** subunit B (EtxB) or **cholera toxin** subunit B (CtxB) to deliver an agent to a target cell wherein the mutant has GM-1 binding activity, and a reduced immunogenic and immunomodulatory activity relative to the wild type form of EtxB or CtxB. Specifically, the mutant CtxB with His to Ala substitution at position 57 is severely defective as an immunomodulator, and the holotoxin exhibits ablated toxicity, and retains the ability to bind with high affinity to GM-1. The invention further discloses that EtxB or an EtxB(H57A) are able to act as trafficking mols. that facilitates delivery of exogenous epitopes into the endogenous pathway of class I antigen processing and presentation.

L49 ANSWER 3 OF 11 SCISEARCH COPYRIGHT (c) 2006 The Thomson Corporation on STN

2003:282407 The Genuine Article (R) Number: 657QM. The B subunit of Escherichia coli heat-labile **enterotoxin** enhances CD8(+) cytotoxic-T-lymphocyte killing of Epstein-Barr virus-infected cell lines. Ong K W; Wilson A D; **Hirst T R**; Morgan A J (Reprint). Univ Bristol, Sch Med Sci, Dept Pathol & Microbiol, Bristol BS8 1TD, Avon, England (Reprint). andy.morgan@bristol.ac.uk. JOURNAL OF VIROLOGY (APR 2003) Vol. 77, No. 7, pp. 4298-4305. ISSN: 0022-538X. Publisher: AMER SOC MICROBIOLOGY, 1752 N ST NW, WASHINGTON, DC 20036-2904 USA. Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Epstein-Barr virus (EBV) is associated with a number of important human cancers, including nasopharyngeal carcinoma, gastric carcinoma, and Hodgkin's lymphoma. These tumors express a viral nuclear antigen, EBV nuclear antigen 1 (EBNA1), which cannot be presented to T cells in a major

histocompatibility complex class I context, and the viral latent membrane proteins (LMPs). Although the LMPs are expressed in these tumors, no effective immune response is made. We report here that exposure to the cholera-like **enterotoxin** B subunit (EtxB) in EBV-infected lymphoblastoid cell lines (LCLs) enhances their susceptibility to killing by LMP-specific CD8(+) cytotoxic T lymphocytes (CTLs) in a HLA class I-restricted manner. CTL killing of LCLs is dramatically increased through both transporter-associated protein-dependent and -independent epitopes after EtxB **treatment**. The use of mutant B subunits revealed that the enhanced susceptibility of LCLs to CTL killing is dependent on the B subunit's interaction with GM, but not its signaling properties. These important findings could underpin the development of novel approaches to treating EBV-associated malignancies and may offer a general approach to increasing the presentation of other tumor and viral antigens.

L49 ANSWER 4 OF 11 MEDLINE on STN DUPLICATE 1

2002150343. PubMed ID: 11882700. Contribution of the ADP-ribosylating and receptor-binding properties of cholera-like **enterotoxins** in modulating cytokine secretion by human intestinal epithelial cells. Soriani Marco; Bailey Lorna; **Hirst Timothy R.** (Department of Pathology and Microbiology, University of Bristol, Bristol BS8 1TD, UK.) Microbiology (Reading, England), (2002 Mar) Vol. 148, No. Pt 3, pp. 667-76. Journal code: 9430468. ISSN: 1350-0872. Pub. country: England: United Kingdom. Language: English.

AB When epithelial cells first encounter **cholera toxin** (Ctx) produced by Vibrio cholerae they secrete not only chloride ions responsible for causing diarrhoea, but also a number of cytokines that may contribute to the **toxin**'s potent immunomodulatory properties. Much less is known about the ability of the heat-labile **enterotoxin** of Escherichia coli (Etx), a close homologue of Ctx, to elicit cytokine secretion by epithelial cells. This study shows that **treatment** of human intestinal epithelial T84 cells with Etx induces expression of IL-6, IL-10, IL-1R antagonist, as well as IL-1alpha and IL-1beta and low levels of IL-8. Such induction was totally dependent on the intrinsic ADP-ribosylating activity of the **toxin** A-subunit, and could be mimicked by cAMP-elevating agents, such as forskolin and dibutyryl cAMP. By comparison, neither an enzymically inactive mutant of Etx nor EtxB was able to induce cytokine secretion. The behaviour of Ctx and CtxB was very similar to that of Etx and EtxB, respectively. The spectrum of cytokines released by Etx and Ctx indicates that the **toxins** may create a local microenvironment that strongly biases the immune response towards an anti-inflammatory and a polarized Th2 response.

L49 ANSWER 5 OF 11 MEDLINE on STN

2003509260. PubMed ID: 14585161. Immune modulation by the cholera-like **enterotoxins**. Salmond Robert J; Luross Jeffrey A; **Williams Neil A.** (The Babraham Institute, Laboratory of Lymphocyte Signalling and Development, Babraham, Cambridge, CB2 4AT, UK.. robert.salmond@bbsrc.ac.uk) . Expert reviews in molecular medicine [electronic resource], (2002 Oct 1) Vol. 2002, pp. 1-16. Electronic Publication: 2002-10-01. Ref: 68. Journal code: 100939725. E-ISSN: 1462-3994. Pub. country: England: United Kingdom. Language: English.

AB The role of **cholera toxin** and heat-labile **enterotoxin** in the pathogenesis of diarrhoeal disease has been well documented for many years. In addition to these deleterious effects, a wealth of data is accumulating that suggests that these **toxins** and their subunits might be used to modulate immune responses in a variety of beneficial ways. In this regard, the **toxins** can boost immune responses to unrelated antigens, leading to the possibility of their use in the generation of improved vaccines to a variety of pathogens. Furthermore, recent evidence suggests that recombinant preparations of the

nontoxic B subunits of the **toxins** have distinct immunomodulatory activities, with potential applications to the **treatment** of autoimmune and inflammatory diseases. This article reviews our current understanding of the mechanisms of immune modulation by these fascinating proteins.

L49 ANSWER 6 OF 11 CAPLUS COPYRIGHT 2006 ACS on STN
2002:862343 Document No. 138:71348 Immune modulation by cholera-like **enterotoxins**. Salmon, Robert J.; Luross, Jeffrey A.; **Williams, Neil A.** (The Babraham Institute, Laboratory of Lymphocyte Signalling and Development, Babraham,Cambridge, CB24AT, UK). Expert Reviews in Molecular Medicine [online computer file] No pp. given (English) 2002. CODEN: ERMMFS. ISSN: 1462-3994. URL: http://www-ermm.cbcu.cam.ac.uk/02005057a.pdf Publisher: Cambridge University Press.
AB A review. The role of **cholera toxin** and heat-labile **enterotoxin** in the pathogenesis of diarrheal disease has been well documented for many years. In addition to these deleterious effects, a wealth of data is accumulating that suggests that these **toxins** and their subunits might be used to modulate immune responses in a variety of beneficial ways. In this regard, the **toxins** can boost immune responses to unrelated antigens, leading to the possibility of their use in the generation of improved vaccines to a variety of pathogens. Furthermore, recent evidence suggests that recombinant prepns. of the nontoxic B subunits of the **toxins** have distinct immunomodulatory activities, with potential applications to the **treatment** of autoimmune and inflammatory diseases. This article reviews the current understanding of the mechanisms of immune modulation by these fascinating proteins.

L49 ANSWER 7 OF 11 CAPLUS COPYRIGHT 2006 ACS on STN
2001:798749 Document No. 135:339267 Therapeutic agents. **Williams, Neil Andrew; Hirst, Timothy Raymond;** Nashar, Toufic Osman (UK). U.S. Pat. Appl. Publ. US 20010036917 A1 20011101, 53 pp., Cont.-in-part of U.S. 6,287,563. (English). CODEN: USXXCO. APPLICATION: US 2001-867914 20010530. PRIORITY: GB 1995-13733 19950705; US 1997-999458 19971229.
AB A method of treating diabetes in a mammalian subject by administering an agent capable of modulating a ganglioside GM-1 (GM-1) associated activity in an amount effect to treat the disease; wherein agent is selected from the group consisting of **cholera toxin** (Ctx), **enterotoxins** (Etx), the B subunit of Ctx and the B subunit of Etx, mutants and derivs. thereof. along with co-administration of antigens which are not so linked to form a single active agent.

L49 ANSWER 8 OF 11 SCISEARCH COPYRIGHT (c) 2006 The Thomson Corporation on STN
2001:600700 The Genuine Article (R) Number: 456UP. Escherichia coli **enterotoxin** B subunit triggers apoptosis of CD8(+) T cells by activating transcription factor c-Myc. Soriani M; **Williams N A; Hirst T R (Reprint)**. Univ Bristol, Dept Pathol & Microbiol, Bristol BS8 1TD, Avon, England (Reprint). INFECTION AND IMMUNITY (AUG 2001) Vol. 69, No. 8, pp. 4923-4930. ISSN: 0019-9567. Publisher: AMER SOC MICROBIOLOGY, 1752 N ST NW, WASHINGTON, DC 20036-2904 USA. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS
AB Heat-labile **enterotoxin** from enterotoxinogenic Escherichia coli is not only an important cause of diarrhea in humans and domestic animals but also possesses potent immunomodulatory properties. Recently, the nontoxic, receptor-binding B subunit of heat-labile **enterotoxin** (EtxB) was found to induce the selective death of CD8(+) T cells, suggesting that EtxB may trigger activation of proapoptotic signaling pathways. Here we show that EtxB **treatment** of CD8(+) T cells but not of CD4(+) T cells triggers the specific

up-regulation of the transcription factor c-myc, implicated in the control of cell proliferation, differentiation, and death. A concomitant elevation in Myc protein levels was also evident, with peak expression occurring 4 h posttreatment. Preincubation with c-myc antisense oligodeoxynucleotides demonstrated that Myc expression was necessary for EtxB-mediated apoptosis. Myc activation was also associated with an increase of I kappaB alpha turnover, suggesting that elevated Myc expression may be dependent on NF-kappaB. When CD8(+) T cells were pretreated with inhibitors of I kappaB alpha turnover and NF-kappaB translocation, this resulted in a marked reduction in both EtxB-induced apoptosis and Myc expression. Further, a non-receptor-binding mutant of EtxB, EtxB(G33D), was shown to lack the capacity to activate Myc transcription. These findings provide further evidence that EtxB is a signaling molecule that triggers activation of transcription factors involved. in cell survival.

L49 ANSWER 9 OF 11 MEDLINE on STN DUPLICATE 2
2001210820. PubMed ID: 11298654. **Cholera toxin** and Escherichia coli **enterotoxin** B-subunits inhibit macrophage-mediated antigen processing and presentation: evidence for antigen persistence in non-acidic recycling endosomal compartments. Millar D G; **Hirst T R.** (Department of Pathology and Microbiology, University of Bristol, School of Medical Sciences, Bristol BS8 1TD, UK.) Cellular microbiology, (2001 May) Vol. 3, No. 5, pp. 311-29. Journal code: 100883691. ISSN: 1462-5814. Pub. country: England: United Kingdom. Language: English.

AB **Cholera toxin** (Ctx) and the closely related Escherichia coli heat-labile **enterotoxin** (Etx) not only act as mediators of diarrhoeal disease but also exert potent immunomodulatory properties on mammalian immune systems. The **toxins** normally exert their diarrhoeagenic effects by initiating receptor-mediated uptake into vesicles that enter a retrograde trafficking pathway, circumventing degradative compartments and targeting them to the trans-Golgi network (TGN) and endoplasmic reticulum. Here, we examine whether receptor-mediated binding and cellular entry by the **toxin** B-subunits also lead to concomitant changes in uptake and trafficking of exogenous antigens that could contribute to the potent immunomodulatory properties of these **toxins**. **Treatment** of the macrophage (J774.2) cell line with Etx B-subunit (EtxB) resulted in EtxB transport to the TGN and also led to the formation of large, translucent, non-acidic, EtxB-devoid vacuoles. When exogenous antigens were added, EtxB-treated cells were found to be proficient in both internalization of ovalbumin (OVA) and phagocytosis of bacterial particles. However, the internalized OVA, instead of trafficking along a lysosome-directed endocytic pathway via acidified endosomes, persisted in a non-acidic, light-density compartment that was distinct from the translucent vacuoles. The rerouted OVA did not co-localize with the endosomal markers rab5 or rab11, nor with EtxB, but was retained in a transferrin receptor-positive compartment. The failure of OVA to enter the late endosomal/lysosomal compartments correlated with a striking inhibition of OVA peptide processing and presentation to OVA-responsive CD4+ T-cells. CtxB also modulated OVA trafficking and inhibited antigen presentation. These findings demonstrate that the B-subunits of Ctx and Etx alter the progression of exogenous antigens along the endocytic processing pathway, and prevent or delay efficient epitope presentation and T-cell stimulation. The formation of such 'antigen depots' could contribute to the immunomodulatory properties of these bacterial virulence determinants.

L49 ANSWER 10 OF 11 CAPLUS COPYRIGHT 2006 ACS on STN
2000:175834 Document No. 132:217136 Peptide fragments of **cholera toxin** B or **enterotoxin** B as immunomodulators and vaccine adjuvants and for the **treatment** of **toxin**-induced diarrhea. **Williams, Neil Andrew; Hirst, Timothy Raymond**

(University of Bristol, UK). PCT Int. Appl. WO 2000014114 A1 20000316, 62 pp. DESIGNATED STATES: W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1999-GB2970 19990907. PRIORITY: GB 1998-19484 19980907.

AB A substance is provided which comprises any one or more of an amino acid sequence EVPGSQH, or a variant, homolog, fragment, derivative, or mimetic thereof. The substance is capable of acting in a manner that is the same as or is similar to **enterotoxin** B and/or **cholera toxin** B, but does not exhibit GM-1 binding activity. The substance may be used as an immunomodulator or vaccine adjuvant or for the **treatment** of **toxin**-induced diarrhea.

L49 ANSWER 11 OF 11 CAPLUS COPYRIGHT 2006 ACS on STN

1997:181160 Document No. 126:170385 Therapeutic agents and autoimmune diseases. **Williams, Neil Andrew; Hirst, Timothy Raymond** ; Nashar, Toufic Osman (University of Bristol, UK; Williams Neil Andrew). PCT Int. Appl. WO 9702045 A1 19970123, 62 pp. DESIGNATED STATES: W: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, NL, PT, SE. (English). CODEN: PIXXD2. APPLICATION: WO 1996-GB1614 19960705. PRIORITY: GB 1995-13733 19950705.

AB There is disclosed the use, as an agent in the **treatment** or the prevention of an autoimmune disease, of: (i) an agent having GM-1 binding activity, other than Ctx or Etx, or the B subunits of Ctx and Etx; or (ii) an agent having an effect on GM-1 mediated intracellular signalling events, but no GM-1 binding activity. These agents may also be used in the **treatment** of human T cell leukemia, in the prevention of transplant rejection or GVHD or in a vaccination method for vaccinating a mammalian subject.

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=> s l47 and allergen
L50             0 L47 AND ALLERGEN

=> s l47 and antigen
L51            63 L47 AND ANTIGEN

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L52 ANSWER 1 OF 35 SCISEARCH COPYRIGHT (c) 2006 The Thomson Corporation on STN

2004:879656 The Genuine Article (R) Number: 857QS. The B subunit of Escherichia coli heat-labile **enterotoxin** induces both caspase-dependent and -independent cell death pathways in CD8(+) T cells. Salmond R J; Williams R; **Hirst T R; Williams N A (Reprint)**. Univ Bristol, Sch Med Sci, Dept Pathol & Microbiol, Univ Walk, Bristol BS8 1TD, Avon, England (Reprint); Univ Bristol, Sch Med Sci, Dept Pathol & Microbiol, Bristol BS8 1TD, Avon, England. neil.a.williams@bris.ac.uk. INFECTION AND IMMUNITY (OCT 2004) Vol. 72, No. 10, pp. 5850-5857. ISSN: 0019-9567. Publisher: AMER SOC MICROBIOLOGY, 1752 N ST NW, WASHINGTON, DC 20036-2904 USA. Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The nontoxic B subunit of Escherichia coli beat-labile **enterotoxin** (EtxB) is a potent immunomodulatory molecule that acts both as an adjuvant and to stimulate immune deviation processes, resulting in the suppression of Th1-associated inflammatory responses. The ability of EtxB to alter immune reactivity is dependent on its ability to modulate immune cell function through binding to cell surface molecules, the principal receptor of which is the ubiquitous G(M1)-ganglioside. EtxB activates B cells and **antigen**-presenting cells and induces the selective apoptosis of murine CD8(+) T cells. We postulated that these effects are mediated by the induction of intracellular signaling pathways following EtxB-receptor interaction. We have previously shown that CD8(+) T-cell apoptosis induced by EtxB results from the activation of the transcription factor NF-kappaB and caspases. Here we report that while caspase activity is required for apoptosis, additional features of cell death are caspase independent. EtxB induces a rapid loss of mitochondrial membrane potential and cell viability that are unaffected by caspase inhibitors. In addition, our data suggest that these processes are independent of the activity of Bax and Bcl-2 but are mediated by nitric oxide synthase.

L52 ANSWER 2 OF 35 SCISEARCH COPYRIGHT (c) 2006 The Thomson Corporation on STN

2004:593548 The Genuine Article (R) Number: 832QU. Nasal delivery of **antigen** with the B subunit of Escherichia coli heat-labile **enterotoxin** augments **antigen**-specific T-cell clonal expansion and differentiation. Apostolaki M; **Williams N A (Reprint)**. Univ Bristol, Sch Med Sci, Dept Pathol & Microbiol, Univ Walk, Bristol, Avon, England (Reprint); Univ Bristol, Sch Med Sci, Dept Pathol & Microbiol, Bristol, Avon, England. neil.a.williams@bris.ac.uk. INFECTION AND IMMUNITY (JUL 2004) Vol. 72, No. 7, pp. 4072-4080. ISSN: 0019-9567. Publisher: AMER SOC MICROBIOLOGY, 1752 N ST NW, WASHINGTON, DC 20036-2904 USA. Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Escherichia coli heat-labile **enterotoxin** has unique immunogenic and adjuvant properties when administered mucosally to mice. These properties have revealed the potential for its use in the development of mucosal vaccines, an area of increasing interest. However, the inherent toxicity mediated by the A subunit precludes its widespread use. This problem has led to attempts to dissociate toxicity from adjuvant function by use of the B subunit. The ability of the B subunit of E. coli heat-labile **enterotoxin** (EtxB) to enhance responses against **antigens** coadministered intranasally is demonstrated here with the use of the DO11.10 adoptive-transfer model, in which ovalbumin (OVA)-specific adoptively transferred T cells can be monitored directly by How cytometry. Intranasal delivery of OVA with EtxB resulted in increased T-cell proliferative and systemic antibody responses against **antigens**. The increased Th2 cytokine production detected following in vitro restimulation of splenocyte and cervical lymph node (CLN) cells from the immunized mice correlated with increased OVA-specific immunoglobulin G1 antibody production. Flow cytometric analysis of T cells from mice early after immunization directly revealed the ability of EtxB to support **antigen**-specific clonal expansion and differentiation. Furthermore, while responses were first detected in the CLNs, they rapidly progressed to the spleen, where they were further sustained. Examination of CD69 expression on dividing cells supported the notion that activation induced by the presence of **antigens** is not sufficient to drive T-cell differentiation. Furthermore, a lack of CD25 expression on dividing cells suggested that EtxB-mediated T-cell clonal expansion may occur without a sustained requirement for interleukin 2.

L52 ANSWER 3 OF 35 MEDLINE on STN DUPLICATE 1

2004077030. PubMed ID: 14965302. Modulation of the immune response by the cholera-like **enterotoxins**. Plant Andrea; **Williams Neil A**. (University of Bristol, Department of Pathology and Microbiology, School of Medical Sciences, University Walk, Bristol, BS8 1TD, UK.) Current topics in medicinal chemistry, (2004) Vol. 4, No. 5, pp. 509-19. Ref: 112. Journal code: 101119673. ISSN: 1568-0266. Pub. country: Netherlands. Language: English.

AB **Cholera toxins** and heat labile **enterotoxin** from E. coli differ from most soluble proteins in eliciting systemic immunity both against themselves and unrelated admixed **antigens**, rather than tolerance following administration to a mucosal surface. Several reports have also demonstrated preferential induction of Th2-type responses when these molecules are used as adjuvants. Conversely, these proteins and their non-toxic derivatives, including the B sub-units are also able prevent and alleviate autoimmune diseases in naive and systemically immune hosts demonstrating wide-ranging effects on the immune system. The recent observation that amelioration of autoimmune disease is associated with the generation of regulatory T cells which inhibit pathogenic Th1 responses may also help to consolidate these two apparently contradictory outcomes of exposure to the cholera-like **enterotoxins**. Furthermore, the observation that EtxB is able to alleviate autoimmune disease in the absence of conjugation to autoantigen highlights its potential for use in the clinical setting where the target **antigen** is often unknown. Direct effects on T cells, B cells and APC have been demonstrated in vitro which have provided insights into how these molecules may elicit these diverse effects. Further investigation is required for elucidation of the mechanisms of action of adjuvanticity and tolerance induction by these molecules to realise their potential for use in vaccines and therapies for autoimmune disease in humans.

L52 ANSWER 4 OF 35 CAPLUS COPYRIGHT 2006 ACS on STN

2003:6139 Document No. 138:68275 Mutant forms of **enterotoxin** (EtxB) and **cholera toxin** (CtxB), and their therapeutic uses as target site-specific carriers. **Hirst, Timothy Raymond** (University of Bristol, UK). PCT Int. Appl. WO 2003000899 A1 20030103, 84 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2002-GB2829 20020620. PRIORITY: GB 2001-15382 20010622.

AB The present invention describes the use of a mutant form of **enterotoxin** subunit B (EtxB) or **cholera toxin** subunit B (CtxB) to deliver an agent to a target cell wherein the mutant has GM-1 binding activity, and a reduced immunogenic and immunomodulatory activity relative to the wild type form of EtxB or CtxB. Specifically, the mutant CtxB with His to Ala substitution at position 57 is severely defective as an immunomodulator, and the holotoxin exhibits ablated toxicity, and retains the ability to bind with high affinity to GM-1. The invention further discloses that EtxB or an EtxB(H57A) are able to act as trafficking mols. that facilitates delivery of exogenous epitopes into the endogenous pathway of class I **antigen** processing and presentation.

L52 ANSWER 5 OF 35 SCISEARCH COPYRIGHT (c) 2006 The Thomson Corporation on STN

2003:282407 The Genuine Article (R) Number: 657QM. The B subunit of Escherichia coli heat-labile **enterotoxin** enhances CD8(+) cytotoxic-T-lymphocyte killing of Epstein-Barr virus-infected cell lines.

Ong K W; Wilson A D; **Hirst T R**; Morgan A J (Reprint). Univ Bristol, Sch Med Sci, Dept Pathol & Microbiol, Bristol BS8 1TD, Avon, England (Reprint). andy.morgan@bristol.ac.uk. JOURNAL OF VIROLOGY (APR 2003) Vol. 77, No. 7, pp. 4298-4305. ISSN: 0022-538X. Publisher: AMER SOC MICROBIOLOGY, 1752 N ST NW, WASHINGTON, DC 20036-2904 USA. Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Epstein-Barr virus (EBV) is associated with a number of important human cancers, including nasopharyngeal carcinoma, gastric carcinoma, and Hodgkin's lymphoma. These tumors express a viral nuclear **antigen** , EBV nuclear **antigen** 1 (EBNA1), which cannot be presented to T cells in a major histocompatibility complex class I context, and the viral latent membrane proteins (LMPs). Although the LMPs are expressed in these tumors, no effective immune response is made. We report here that exposure to the cholera-like **enterotoxin** B subunit (EtxB) in EBV-infected lymphoblastoid cell lines (LCLs) enhances their susceptibility to killing by LMP-specific CD8(+) cytotoxic T lymphocytes (CTLs) in a HLA class I-restricted manner. CTL killing of LCLs is dramatically increased through both transporter-associated protein-dependent and -independent epitopes after EtxB treatment. The use of mutant B subunits revealed that the enhanced susceptibility of LCLs to CTL killing is dependent on the B subunit's interaction with GM, but not its signaling properties. These important findings could underpin the development of novel approaches to treating EBV-associated malignancies and may offer a general approach to increasing the presentation of other tumor and viral **antigens**.

L52 ANSWER 6 OF 35 SCISEARCH COPYRIGHT (c) 2006 The Thomson Corporation on STN

2003:995438 The Genuine Article (R) Number: 741GK. The B subunit of Escherichia coli heat labile **enterotoxin** abrogates oral tolerance, promoting predominantly Th2-type immune responses. Plant A; Williams R; Jackson M E; **Williams N A (Reprint)**. Univ Bristol, Dept Pathol & Microbiol, Sch Med Sci, Univ Walk, Bristol BS8 1TD, Avon, England (Reprint); Univ Bristol, Dept Pathol & Microbiol, Sch Med Sci, Bristol BS8 1TD, Avon, England. EUROPEAN JOURNAL OF IMMUNOLOGY (NOV 2003) Vol. 33, No. 11, pp. 3186-3195. ISSN: 0014-2980. Publisher: WILEY-V C H VERLAG GMBH, PO BOX 10 11 61, D-69451 WEINHEIM, GERMANY. Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Mucosal **antigen** encounter usually results in a state of systemic non-responsiveness (tolerance). This failure to mount a protective response is a major hurdle to mucosal vaccine development. Hence, the identification of safe and effective mucosal adjuvants promoting protective immunity is of critical importance. The non-toxic B subunit of Escherichia coli heat labile **enterotoxin** (EtxB) is a potent nasal adjuvant; however, its usefulness following oral delivery is unconfirmed. We used DO11.10 chimeric mice to assess whether EtxB could abrogate tolerance to oral OVA. We show that admixing EtxB with OVA for oral immunization abrogates oral tolerance and results in a weak anti-OVA immune response. Importantly, EtxB profoundly modulated the nature of the response to subsequent parenteral challenge, promoting IgG1 in favor of IgG2a antibodies and depressing IFN-gamma production while elevating TGF-beta secretion. The addition of EtxB promoted T cell division, as assessed by loss of staining with carboxyfluorescein diacetate succinimidyl ester. Enhanced cell division promoted by EtxB was associated with T cell differentiation (increased numbers of CD45RB(low) cells) in vivo, although dividing OVA-specific T cells were CD25(-). These data suggest that although EtxB is a weak oral adjuvant, it can profoundly modulate the nature of the immune response to admixed **antigen**.

L52 ANSWER 7 OF 35 SCISEARCH COPYRIGHT (c) 2006 The Thomson Corporation on

2003:215856 The Genuine Article (R) Number: 650NU. Mutant Escherichia coli heat-labile **toxin** B subunit that separates toxoid-mediated signaling and immunomodulatory action from trafficking and delivery functions. Fraser S A; de Haan L; Hearn A R; Bone H K; Salmond R J; Rivett A J; **Williams N A; Hirst T R (Reprint)**. Univ Bristol, Sch Med Sci, Dept Pathol & Microbiol, Univ Walk, Bristol BS8 1TD, Avon, England (Reprint); Univ Bristol, Sch Med Sci, Dept Pathol & Microbiol, Bristol BS8 1TD, Avon, England; Univ Bristol, Sch Med Sci, Dept Biochem, Bristol BS8 1TD, Avon, England. INFECTION AND IMMUNITY (MAR 2003) Vol. 71, No. 3, pp. 1527-1537. ISSN: 0019-9567. Publisher: AMER SOC MICROBIOLOGY, 1752 N ST NW, WASHINGTON, DC 20036-2904 USA. Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The homopentameric B-subunit components of Escherichia coli heat-labile **enterotoxin** (EtxB) and **cholera toxin** (CtxB) possess the capacity to enter mammalian cells and to activate cell-signaling events in leukocytes that modulate immune cell function. Both properties have been attributed to the ability of the B subunit's to bind to GM1-ganglioside receptors, a ubiquitous glycosphingolipid found in the plasma membrane. Here we describe the properties of EtxB(H57S), a mutant B subunit with a His-->Ser substitution at position 57. The mutant was found to be severely defective in inducing leukocyte signaling, as shown by failure to (i) trigger caspase 3-mediated CD8(+)-T-cell apoptosis, (ii) activate nuclear translocation of NF-KB in Jurkat T cells, (iii) induce a potent anti-B-subunit response in mice, or (iv) serve as a mucosall adjuvant. However, its GM1 binding, cellular uptake, and delivery functions remained intact. This was further validated by the finding that EtxB(H57S) was as effective as EtxB in delivering a conjugated model class I epitope into the major histocompatibility complex class I pathway of a dendritic cell line. These observations imply that GM1 binding alone is not sufficient to trigger the signaling events responsible for the potent immunomodulatory properties of EtxB. Moreover, they demonstrate that its signaling properties play no role in EtxB uptake and trafficking. Thus, EtxB(H57S) represents a novel tool for evaluating the complex cellular interactions and signaling events occurring after receptor interaction, as well as offering an alternative means of delivering attached peptides in the absence of the potent immunomodullatory signals induced by wild-type B subunits.

L52 ANSWER 8 OF 35 SCISEARCH COPYRIGHT (c) 2006 The Thomson Corporation on STN

2002:457870 The Genuine Article (R) Number: 554XA. Enhanced delivery of exogenous peptides into the class I **antigen** processing and presentation pathway. de Haan L; Hearn A R; Rivett A J; **Hirst T R (Reprint)**. Univ Bristol, Sch Med Sci, Dept Pathol & Microbiol, Univ Walk, Bristol BS8 1TD, Avon, England (Reprint); Univ Bristol, Sch Med Sci, Dept Pathol & Microbiol, Bristol BS8 1TD, Avon, England; Univ Bristol, Sch Med Sci, Dept Biochem, Bristol BS8 1TD, Avon, England. INFECTION AND IMMUNITY (JUN 2002) Vol. 70, No. 6, pp. 3249-3258. ISSN: 0019-9567. Publisher: AMER SOC MICROBIOLOGY, 1752 N ST NW, WASHINGTON, DC 20036-2904 USA. Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Current immunization strategies, using peptide or protein **antigens**, generally fail to elicit cytotoxic-T-lymphocyte responses, since these **antigens** are unable to access intracellular compartments where loading of major histocompatibility complex class I (MHC-I) molecules occurs. In an attempt to circumvent this, we investigated whether the GM1 receptor-binding B subunit of Escherichia coli heat-labile **toxin** (EtxB) could be used to deliver class I epitopes. When a class I epitope was conjugated to EtxB, it was delivered into the MHC-I presentation pathway in a

GM1-binding-dependent fashion and resulted in the appearance of MHC-I-epitope complexes at the cell surface. Importantly, we show that the efficiency of EtxB-mediated epitope delivery could be strikingly enhanced by incorporating, adjacent to the class I epitope, a 10-amino-acid segment from the C terminus of the DNA polymerase (Pol) of herpes simplex virus. The replacement of this 10-amino-acid segment by a heterologous sequence or the introduction of specific amino acid substitutions within this segment either abolished or markedly reduced the efficiency of class I epitope delivery. If the epitope was extended at its C terminus, EtxB-mediated delivery into the class I presentation pathway was found to be completely dependent on proteasome activity. Thus, by combining the GM1-targeting function of EtxB with the 10-amino-acid Pol segment, highly efficient delivery of exogenous epitopes into the endogenous pathway of class I **antigen** processing and presentation can be achieved.

L52 ANSWER 9 OF 35 SCISEARCH COPYRIGHT (c) 2006 The Thomson Corporation on STN

2002:477079 The Genuine Article (R) Number: 557MJ. Modulation of B lymphocyte signalling by the B subunit of Escherichia coli heat-labile **enterotoxin**. Bone H; Eckholdt S; **Williams N A (Reprint)** . Univ Bristol, Sch Med Sci, Dept Pathol & Microbiol, Univ Walk, Bristol BS8 1TD, Avon, England (Reprint); Univ Bristol, Sch Med Sci, Dept Pathol & Microbiol, Bristol BS8 1TD, Avon, England. INTERNATIONAL IMMUNOLOGY (JUN 2002) Vol. 14, No. 6, pp. 647-658. ISSN: 0953-8178. Publisher: OXFORD UNIV PRESS, GREAT CLARENDON ST, OXFORD OX2 6DP, ENGLAND. Language: English. *ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS*

AB The non-toxic B subunit of Escherichia coli heat-labile **enterotoxin** (EtxB) is a potent mucosal adjuvant and immunomodulator capable of blocking autoimmune disease. These effects are linked with its ability to modulate lymphocyte populations-a feature that is dependent on binding to ubiquitously expressed cell surface receptors. Here, we demonstrate that EtxB can trigger up-regulated expression of class II MHC and CD25 on purified populations of B lymphocytes, suggesting that EtxB can directly activate biochemical signalling pathways in these cells. The nature of the intracellular signalling events was investigated. B cells cultured with EtxB, but not a non-receptor binding mutant protein, EtxB(G33D), caused the activation of the extracellular signal-regulated kinase (Erk) forms of mitogen-activated protein (MAP) kinase in a process that was dependent on MAPK/Erk kinase (MEK), phosphoinositide 3-kinase (PI3-kinase) and protein kinase C (PKC), as determined by the use of specific inhibitors. PI3-kinase was critical not only in the activation of MAP kinase but also in the up-regulation of both class II and CD25. However, MEK inhibition only partially abrogated the EtxB-mediated up-regulation of MHC class II expression and did not affect CD25 expression-findings suggesting that additional pathways downstream of PI3-kinase are involved. A role for PKC in these processes was suggested by the finding that inhibitors of PKC completely blocked EtxB-mediated CD25 up-regulation. Thus, we have shown that receptor binding by EtxB triggers multiple signalling pathways in B cells that regulate the expression of key cell surface molecules.

L52 ANSWER 10 OF 35 SCISEARCH COPYRIGHT (c) 2006 The Thomson Corporation on STN

2002:569643 The Genuine Article (R) Number: 568CX. Modulation of human monocytes by Escherichia coli heat-labile **enterotoxin** B-subunit; altered cytokine production and its functional consequences. Turcanu V; **Hirst T R; Williams N A (Reprint)**. Univ Bristol, Dept Pathol & Microbiol, Sch Med Sci, Univ Walk, Bristol BS8 1TD, Avon, England (Reprint); Univ Bristol, Dept Pathol & Microbiol, Sch Med Sci, Bristol BS8 1TD, Avon, England. IMMUNOLOGY (JUL 2002) Vol. 106, No. 3, pp. 316-325. ISSN: 0019-2805. Publisher: BLACKWELL PUBLISHING LTD, 9600 GARSINGTON RD, OXFORD OX4 2DG, OXON, ENGLAND. Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB In murine systems, the B subunit of Escherichia coli heat-labile **enterotoxin** (EtxB) is a potent immunomodulator capable of suppressing Th1-mediated autoimmune diseases. This results from its ability to bind cell surface receptors, principally GM1-ganglioside, and as a consequence down-regulate the pathological T helper type 1 (Th1) response. The capacity of EtxB to alter human T-cell responses has not been investigated. Here we show that EtxB, but not the receptor non-binding mutant EtxB (G33D), triggers the release of interleukin (IL)-10, IL-6 and tumour necrosis factor-alpha (TNF-alpha) by human monocytes. The production of IL-8, transforming growth factor-beta (TGF-beta) or IL-12 was not enhanced by EtxB. Indeed, EtxB was shown to inhibit IL-12 secretion in monocytes stimulated with interferon-gamma (IFN-gamma) and lipopolysaccharide (LPS) by an IL-10-independent mechanism. When EtxB-treated monocytes were used as **antigen** presenting cells in an allogeneic mixed lymphocyte reaction (MLR), IL-10 and IFN-gamma production were increased in comparison to levels seen in cultures stimulated with untreated monocytes; proliferation was unaltered. This modulation of the T-cell response was not only evident in the primary MLR triggered by EtxB-treated monocytes, but also upon restimulation of the responding T cells with fresh untreated monocytes; indicating that presentation by EtxB-treated monocytes leads to altered T-cell differentiation. Sorting experiments showed that IL-10 secreting T cells from the MLR cultures were strong suppressors of T-cell proliferation following their addition into a fresh primary MLR.

L52 ANSWER 11 OF 35 MEDLINE on STN

2003509260. PubMed ID: 14585161. Immune modulation by the cholera-like **enterotoxins**. Salmond Robert J; Luross Jeffrey A; **Williams Neil A.** (The Babraham Institute, Laboratory of Lymphocyte Signalling and Development, Babraham, Cambridge, CB2 4AT, UK.. robert.salmond@bbsrc.ac.uk) . Expert reviews in molecular medicine [electronic resource], (2002 Oct 1) Vol. 2002, pp. 1-16. Electronic Publication: 2002-10-01. Ref: 68. Journal code: 100939725. E-ISSN: 1462-3994. Pub. country: England: United Kingdom. Language: English.

AB The role of **cholera toxin** and heat-labile **enterotoxin** in the pathogenesis of diarrhoeal disease has been well documented for many years. In addition to these deleterious effects, a wealth of data is accumulating that suggests that these **toxins** and their subunits might be used to modulate immune responses in a variety of beneficial ways. In this regard, the **toxins** can boost immune responses to unrelated **antigens**, leading to the possibility of their use in the generation of improved vaccines to a variety of pathogens. Furthermore, recent evidence suggests that recombinant preparations of the nontoxic B subunits of the **toxins** have distinct immunomodulatory activities, with potential applications to the treatment of autoimmune and inflammatory diseases. This article reviews our current understanding of the mechanisms of immune modulation by these fascinating proteins.

L52 ANSWER 12 OF 35 CAPLUS COPYRIGHT 2006 ACS on STN

2002:862343 Document No. 138:71348 Immune modulation by cholera-like **enterotoxins**. Salmon, Robert J.; Luross, Jeffrey A.; **Williams, Neil A.** (The Babraham Institute, Laboratory of Lymphocyte Signalling and Development, Babraham,Cambridge, CB24AT, UK). Expert Reviews in Molecular Medicine [online computer file] No pp. given (English) 2002. CODEN: ERMMFS. ISSN: 1462-3994. URL: http://www-ermm.cbcu.cam.ac.uk/02005057a.pdf Publisher: Cambridge University Press.

AB A review. The role of **cholera toxin** and heat-labile **enterotoxin** in the pathogenesis of diarrheal disease has been well documented for many years. In addition to these deleterious effects, a wealth of data is accumulating that suggests that these **toxins**

and their subunits might be used to modulate immune responses in a variety of beneficial ways. In this regard, the **toxins** can boost immune responses to unrelated **antigens**, leading to the possibility of their use in the generation of improved vaccines to a variety of pathogens. Furthermore, recent evidence suggests that recombinant prepns. of the nontoxic B subunits of the **toxins** have distinct immunomodulatory activities, with potential applications to the treatment of autoimmune and inflammatory diseases. This article reviews the current understanding of the mechanisms of immune modulation by these fascinating proteins.

L52 ANSWER 13 OF 35 CAPLUS COPYRIGHT 2006 ACS on STN
2001:798749 Document No. 135:339267 Therapeutic agents. **Williams, Neil Andrew; Hirst, Timothy Raymond;** Nashar, Toufic Osman (UK). U.S. Pat. Appl. Publ. US 20010036917 A1 20011101, 53 pp., Cont.-in-part of U.S. 6,287,563. (English). CODEN: USXXCO. APPLICATION: US 2001-867914 20010530. PRIORITY: GB 1995-13733 19950705; US 1997-999458 19971229.
AB A method of treating diabetes in a mammalian subject by administering an agent capable of modulating a ganglioside GM-1 (GM-1) associated activity in an amount effect to treat the disease; wherein agent is selected from the group consisting of **cholera toxin** (Ctx), **enterotoxins** (Etx), the B subunit of Ctx and the B subunit of Etx, mutants and derivs. thereof. along with co-administration of **antigens** which are not so linked to form a single active agent.

L52 ANSWER 14 OF 35 SCISEARCH COPYRIGHT (c) 2006 The Thomson Corporation on STN
2001:338101 The Genuine Article (R) Number: 423CT. Escherichia coli heat-labile **enterotoxin** B subunit is a more potent mucosal adjuvant than its closely related homologue, the B subunit of **cholera toxin**. Millar D G (Reprint); **Hirst T R** ; Snider D P. Ontario Canc Inst, Dept Med Biophys, 610 Univ Ave, Room 8-318, Toronto, ON M5G 2M9, Canada (Reprint); McMaster Univ, Dept Pathol & Mol Med, Hamilton, ON L8N 3Z5, Canada; Univ Bristol, Dept Pathol & Microbiol, Bristol, Avon, England. INFECTION AND IMMUNITY (MAY 2001) Vol. 69, No. 5, pp. 3476-3482. ISSN: 0019-9567. Publisher: AMER SOC MICROBIOLOGY, 1752 N ST NW, WASHINGTON, DC 20036-2904 USA. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS
AB Although **cholera toxin** (Ch) and Escherichia coli heat-labile **enterotoxin** (Etx) are known to be potent mucosal adjuvants, it remains controversial whether the adjuvanticity of the holotoxins extends to their nontoxic, receptor-binding B subunits, Here, ave halle systematically evaluated the comparative adjuvant properties of highly purified recombinant EtxB and CtxB, EtxB was found to be a more potent adjuvant than CtxB, stimulating responses to hell egg lysozyme when the two were coadministered to mice intranasally, as assessed by enhanced serum and secretory antibody titers as well as by stimulation of lymphocyte proliferation in spleen and draining lymph nodes. These results indicate that, although structurally very similar, EtxB and CtxB have strikingly different immunostimulatory propel ties and should not be considered equivalent as prospective vaccine adjuvants.

L52 ANSWER 15 OF 35 SCISEARCH COPYRIGHT (c) 2006 The Thomson Corporation on STN
2001:122816 The Genuine Article (R) Number: 397LK. Protective mucosal immunity to ocular herpes simplex virus type 1 infection in mice by using Escherichia coli heat-labile **enterotoxin** B subunit as an adjuvant. Richards C M (Reprint); Aman A T; **Hirst T R**; Hill T J; **Williams N A**. Univ Bristol, Sch Med Sci, Dept Pathol & Microbiol, Univ Walk, Bristol BS8 1TD, Avon, England (Reprint); Univ Bristol, Sch Med Sci, Dept Pathol & Microbiol, Bristol BS8 1TD, Avon, England. JOURNAL OF VIROLOGY (FEB 2001) Vol. 75, No. 4, pp. 1664-1671.

ISSN: 0022-538X. Publisher: AMER SOC MICROBIOLOGY, 1752 N ST NW, WASHINGTON, DC 20036-2904 USA. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The potential of nontoxic recombinant B subunits of **cholera toxin** (rCtxB) and its close relative Escherichia coli heat-labile **enterotoxin** (rEtxB) to act as mucosal adjuvants for intranasal immunization with herpes simplex virus type 1 (HSV-1) glycoproteins was assessed. Doses of 10 mug of rEtxB or above with 10 mug of HSV-1 glycoproteins elicited high serum and mucosal anti-HSV-1 titers comparable with that obtained using CtxB (10 mug) with a trace (0.5 mug) of whole **toxin** (Ctx-CtxB). By contrast, doses of rCtxB up to 100 mug elicited only meager anti-HSV-1 responses. As for Ctx-CtxB, rEtxB resulted in a Th2 biased immune response with high immunoglobulin G1 (IgG1)/IgG2a antibody ratios and production of interleukin 4 (IL-4) and IL-10 as well as gamma interferon by proliferating T cells. The protective efficacy of the immune response induced using rEtxB as an adjuvant was assessed following ocular challenge of immunized and mock-immunized mice. Epithelial disease was observed in both groups, but the immunized mice recovered by day 6 whereas mock-immunized mice developed more severe corneal disease leading to stromal keratitis. In addition, a significant reduction in the incidence of lid disease and zosteriform spread was observed in immunized animals and there was no encephalitis compared with 95% encephalitis in mock-immunized mice. The potential of such mucosal adjuvants for use in human vaccines against pathogens such as HSV-1 is discussed.

L52 ANSWER 16 OF 35 MEDLINE on STN DUPLICATE 3

2001210820. PubMed ID: 11298654. **Cholera toxin** and Escherichia coli **enterotoxin** B-subunits inhibit macrophage-mediated **antigen** processing and presentation: evidence for **antigen** persistence in non-acidic recycling endosomal compartments. Millar D G; **Hirst T R.** (Department of Pathology and Microbiology, University of Bristol, School of Medical Sciences, Bristol BS8 1TD, UK.) Cellular microbiology, (2001 May) Vol. 3, No. 5, pp. 311-29. Journal code: 100883691. ISSN: 1462-5814. Pub. country: England: United Kingdom. Language: English.

AB **Cholera toxin** (Ctx) and the closely related Escherichia coli heat-labile **enterotoxin** (Etx) not only act as mediators of diarrhoeal disease but also exert potent immunomodulatory properties on mammalian immune systems. The **toxins** normally exert their diarrhoeagenic effects by initiating receptor-mediated uptake into vesicles that enter a retrograde trafficking pathway, circumventing degradative compartments and targeting them to the trans-Golgi network (TGN) and endoplasmic reticulum. Here, we examine whether receptor-mediated binding and cellular entry by the **toxin** B-subunits also lead to concomitant changes in uptake and trafficking of exogenous **antigens** that could contribute to the potent immunomodulatory properties of these **toxins**. Treatment of the macrophage (J774.2) cell line with Etx B-subunit (EtxB) resulted in EtxB transport to the TGN and also led to the formation of large, translucent, non-acidic, EtxB-devoid vacuoles. When exogenous **antigens** were added, EtxB-treated cells were found to be proficient in both internalization of ovalbumin (OVA) and phagocytosis of bacterial particles. However, the internalized OVA, instead of trafficking along a lysosome-directed endocytic pathway via acidified endosomes, persisted in a non-acidic, light-density compartment that was distinct from the translucent vacuoles. The rerouted OVA did not co-localize with the endosomal markers rab5 or rab11, nor with EtxB, but was retained in a transferrin receptor-positive compartment. The failure of OVA to enter the late endosomal/lysosomal compartments correlated with a striking inhibition of OVA peptide processing and presentation to OVA-responsive CD4+ T-cells. CtxB also modulated OVA trafficking and inhibited **antigen** presentation. These findings demonstrate that the

B-subunits of Ctx and Etx alter the progression of exogenous **antigens** along the endocytic processing pathway, and prevent or delay efficient epitope presentation and T-cell stimulation. The formation of such '**antigen** depots' could contribute to the immunomodulatory properties of these bacterial virulence determinants.

L52 ANSWER 17 OF 35 MEDLINE on STN DUPLICATE 4

2001206764. PubMed ID: 11251877. Immunomodulation using bacterial **enterotoxins**. Simmons C P; Ghaem-Magami M; Petrovska L; Lopes L; Chain B M; **Williams N A**; Dougan G. (Department of Biochemistry, Imperial College of Science Technology and Medicine, South Kensington, London SW7 2AZ, UK.. c.simmons@ic.ac.uk) . Scandinavian journal of immunology, (2001 Mar) Vol. 53, No. 3, pp. 218-26. Ref: 60. Journal code: 0323767. ISSN: 0300-9475. Pub. country: England: United Kingdom. Language: English.

AB Immunologic unresponsiveness (tolerance) is a key feature of the mucosal immune system, and deliberate vaccination by a mucosal route can effectively induce immune suppression. However, some bacterial-derived proteins, e.g. **cholera toxin** and the heat labile **toxin** of Escherichia coli, are immunogenic and immunomodulatory at mucosal surfaces and can effectively adjuvant immune responses to codelivered bystander **antigens**. This review summarizes some of the structural and biological characteristics of these **toxins** and provides examples of how these properties have been exploited for tolerance induction and mucosal vaccine development.

L52 ANSWER 18 OF 35 CAPLUS COPYRIGHT 2006 ACS on STN

2000:175834 Document No. 132:217136 Peptide fragments of **cholera toxin** B or **enterotoxin** B as immunomodulators and vaccine adjuvants and for the treatment of **toxin**-induced diarrhea. **Williams, Neil Andrew; Hirst, Timothy Raymond** (University of Bristol, UK). PCT Int. Appl. WO 2000014114 A1 20000316, 62 pp. DESIGNATED STATES: W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1999-GB2970 19990907. PRIORITY: GB 1998-19484 19980907.

AB A substance is provided which comprises any one or more of an amino acid sequence EVPGSQH, or a variant, homolog, fragment, derivative, or mimetic thereof. The substance is capable of acting in a manner that is the same as or is similar to **enterotoxin** B and/or **cholera toxin** B, but does not exhibit GM-1 binding activity. The substance may be used as an immunomodulator or vaccine adjuvant or for the treatment of **toxin**-induced diarrhea.

L52 ANSWER 19 OF 35 SCISEARCH COPYRIGHT (c) 2006 The Thomson Corporation on STN

2000:853131 The Genuine Article (R) Number: 371LB. Immune modulation by the cholera-like **enterotoxin** B-subunits: from adjuvant to immunotherapeutic. **Williams N A (Reprint)**. Univ Bristol, Sch Med Sci, Dept Pathol & Microbiol, Bristol BS8 1TD, Avon, England (Reprint) . INTERNATIONAL JOURNAL OF MEDICAL MICROBIOLOGY (OCT 2000) Vol. 290, No. 4-5, pp. 447-453. ISSN: 1438-4221. Publisher: URBAN & FISCHER VERLAG, BRANCH OFFICE JENA, P O BOX 100537, D-07705 JENA, GERMANY. Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB **Cholera toxin** (Ctx) and its close relative, Esrherichia coli heat-labile **enterotoxin** (Etx) have long been established as potent mucosal and systemic adjuvants. Problems arising

from their inherent toxicity have, however, precluded human use. Here we describe findings which demonstrate that contrary to the established dogma the non-toxic B-subunit of Etx (EtxB) is a highly potent mucosal adjuvant capable of potentiating protective immunity to viral infection. The mechanisms which underlie this activity arise from an ability to trigger specific signaling processes in lymphocyte populations which modulate differentially their activation, differentiation and survival. The elucidation of these properties has led to the further use of EtxB as an agent capable of preventing the establishment of autoimmune diseases. The basis for these activities and their potential applicability to human therapies are discussed.

L52 ANSWER 20 OF 35 MEDLINE on STN DUPLICATE 5
2000445484. PubMed ID: 10994530. **Cholera toxin** and related **enterotoxins**: a cell biological and immunological perspective. de Haan L; **Hirst T R**. (Department of Pathology and Microbiology, School of Medical Sciences, University of Bristol, United Kingdom.) Journal of natural toxins, (2000 Aug) Vol. 9, No. 3, pp. 281-97. Ref: 126. Journal code: 9208016. ISSN: 1058-8108. Pub. country: United States. Language: English.

AB **Cholera toxin** (Ctx) from Vibrio cholerae and the closely related Escherichia coli heat-labile **enterotoxin** (Etx) are the primary virulence factors responsible for causing cholera and traveller's diarrhea, respectively. Studies on the mode of action of these **toxins** on gut epithelial cells have revealed important insights into the mechanisms of **toxin** uptake and trafficking in eukaryotic cells. However, of perhaps even greater fascination have been the discoveries that Ctx and Etx exhibit remarkable immunological properties. When either of these **toxins** is administered via mucosal routes, it triggers a potent mucosal and systemic anti-**toxin** immune response. By contrast, local or systemic immunization with other soluble protein **antigens** usually stimulates only a meagre immune response, or results in a state of immunological tolerance. Even more striking are the findings that when Ctx or Etx are mixed with heterologous **antigens**, they function as adjuvants, leading to stimulation of mucosal responses to the admixed **antigen**, and the abrogation of oral tolerance. In addition, recent observations have shown that the receptor-binding component of these **toxins** can down-regulate inflammatory diseases associated with the induction of autoimmune disorders such as rheumatoid arthritis, diabetes, and multiple sclerosis. While the underlying mechanisms responsible for these remarkable properties have yet to be resolved, it is clear that the **toxins**' ability to bind to cell surface receptors plays an important role in their potent immunogenicity, adjuvanticity, and immunotherapeutic properties. This review provides an overview of the latest developments within the Ctx/Etx field, with a special emphasis on the cell entry mechanisms and immunomodulatory action of Ctx/Etx and their component subunits.

L52 ANSWER 21 OF 35 CAPLUS COPYRIGHT 2006 ACS on STN
1999:736498 Document No. 131:335799 Immunomodulatory activity of B subunits of **cholera toxin**, verotoxin, and heat-labile **enterotoxin**. **Hirst, Timothy Raymond; Williams, Neil Andrew** (University of Bristol, UK). PCT Int. Appl. WO 9958145 A2 19991118, 63 pp. DESIGNATED STATES: W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1999-GB1461 19990510. PRIORITY: GB 1998-9958 19980508; GB 1998-11954 19980603; GB 1998-12316

19980608.

AB The authors disclose the use of: (i) heat-labile **enterotoxin** B subunit (EtxB), **cholera toxin** B subunit (CtxB) or verotoxin B subunit (VtxB) in vaccine prepns. to alter the immune response to pathogens. In one example, the secretory IgA response to herpes virus glycoproteins is enhanced by the adjuvant activity of EtxB. In addition, the authors disclose the use of agents other than EtxB or CtxB, which have ganglioside GM1-binding activity, or an agent other than VtxB which has globotriosylceramide (Gb3)-binding activity for affecting intracellular signaling events.

L52 ANSWER 22 OF 35 CAPLUS COPYRIGHT 2006 ACS on STN

1999:451202 Document No. 131:82960 EtxB or ganglioside GM1 for treating allergic or hypersensitivity conditions. **Williams, Neil Andrew; Hirst, Timothy Raymond; Bienenstock, John** (Oratol Limited, UK). PCT Int. Appl. WO 9934817 A1 19990715, 46 pp. DESIGNATED STATES: W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1999-GB70 19990108. PRIORITY: GB 1998-487 19980109.

AB The use of an agent in the manufacture of a medicament to treat an allergic condition and/or a hypersensitivity condition is described. The agent is capable of modulating a ganglioside-associated activity. The agent is not coupled to an **antigen**. The modulation of the ganglioside-associated activity affects an allergic condition and/or a hypersensitivity condition. Examples of such modulators include ganglioside GM1 and E. coli **enterotoxin** B subunit.

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1999105073 EMBASE Immune modulation by the cholera-like **enterotoxins** : From adjuvant to therapeutic. **Williams N.A.; Hirst T.R.**; Nashar T.O.. N.A. Williams, University of Bristol, Dept. of Pathology and Microbiology, School of Medical Sciences, Bristol BS8 1TD, United Kingdom. neil.a.william@bris.ac.uk. Immunology Today Vol. 20, No. 2, pp. 95-101 1999.
Refs: 63.
ISSN: 0167-5699. CODEN: IMTOD8
S 0167-5699(98)01397-8. Pub. Country: United Kingdom. Language: English. Summary Language: English.
Entered STN: 19990429. Last Updated on STN: 19990429

AB **Cholera toxin** and its close relative, Escherichia coli heat-labile **enterotoxin**, are potent immunogens and mucosal adjuvants. The recent findings that their B subunits can promote tolerance highlights the complexity of their interactions with the immune system. Here, Neil Williams and colleagues review the mechanisms by which these molecules and seek to explain the paradox.

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1998294533 EMBASE **Cholera toxin** and related **enterotoxins** as potent immune modulators. **Hirst T.R.**; Nashar T.O.; Pitman R.S.; **Williams N.A.**. Prof. T.R. Hirst, University of Bristol, Department of Pathology/Microbiology, School of Medical Sciences, Bristol BS8 1TD, United Kingdom. t.r.hirsrt@bristol.ac.uk. Journal of Applied Microbiology Symposium Supplement Vol. 84, No. 27, pp. 26S-34S 1998.
Refs: 65.
ISSN: 0267-4440. CODEN: SAPBB7

Pub. Country: United Kingdom. Language: English.
Entered STN: 19980917. Last Updated on STN: 19980917
DATA NOT AVAILABLE FOR THIS ACCESSION NUMBER

L52 ANSWER 25 OF 35 SCISEARCH COPYRIGHT (c) 2006 The Thomson Corporation on STN DUPLICATE 7

1998:672661 The Genuine Article (R) Number: 114KQ. Importance of receptor binding in the immunogenicity, adjuvanticity and therapeutic properties of **cholera toxin** and Escherichia coli heat-labile **enterotoxin**. Nashar T O (Reprint); **Williams N A**; **Hirst T R**. Univ Bristol, Sch Med Sci, Dept Pathol & Microbiol, Bristol BS8 1TD, Avon, England (Reprint). MEDICAL MICROBIOLOGY AND IMMUNOLOGY (JUN 1998) Vol. 187, No. 1, pp. 3-10. ISSN: 0300-8584. Publisher: SPRINGER-VERLAG, 175 FIFTH AVE, NEW YORK, NY 10010 USA. Language: English.

L52 ANSWER 26 OF 35 CAPLUS COPYRIGHT 2006 ACS on STN

1997:181160 Document No. 126:170385 Therapeutic agents and autoimmune diseases. **Williams, Neil Andrew; Hirst, Timothy Raymond** ; Nashar, Toufic Osman (University of Bristol, UK; Williams Neil Andrew). PCT Int. Appl. WO 9702045 A1 19970123, 62 pp. DESIGNATED STATES: W: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, NL, PT, SE. (English). CODEN: PIXXD2. APPLICATION: WO 1996-GB1614 19960705. PRIORITY: GB 1995-13733 19950705.

AB There is disclosed the use, as an agent in the treatment or the prevention of an autoimmune disease, of: (i) an agent having GM-1 binding activity, other than Ctx or Etx, or the B subunits of Ctx and Etx; or (ii) an agent having an effect on GM-1 mediated intracellular signalling events, but no GM-1 binding activity. These agents may also be used in the treatment of human T cell leukemia, in the prevention of transplant rejection or GVHD or in a vaccination method for vaccinating a mammalian subject.

L52 ANSWER 27 OF 35 MEDLINE on STN DUPLICATE 8

1998018503. PubMed ID: 9378497. Modulation of B-cell activation by the B subunit of Escherichia coli **enterotoxin**: receptor interaction up-regulates MHC class II, B7, CD40, CD25 and ICAM-1. Nashar T O; **Hirst T R; Williams N A.** (School of Medical Sciences, University of Bristol, UK.) Immunology, (1997 Aug) Vol. 91, No. 4, pp. 572-8. Journal code: 0374672. ISSN: 0019-2805. Pub. country: ENGLAND: United Kingdom. Language: English.

AB The B subunits of **cholera toxin** (CtxB) and Escherichia coli heat-labile **enterotoxin** (EtxB) are non-toxic lectins that bind and cross-link a ubiquitous cell glycolipid receptor, ganglioside GM1, and are recognized as potent mucosal and systemic immunogens. Here we examine the role of EtxB receptor occupancy in modulating the activation of B cells, in vitro, in primary lymphocyte cultures containing B and T cells. When 48-hr spleen cell cultures containing EtxB were compared with those in the presence of a non-receptor binding mutant, EtxB(G33D), a marked shift in the ratio of CD4+ T cells: B cells was noted. Evidence suggested that this was the result of either enhanced survival or proliferation of B cells associated with receptor occupancy by EtxB. Investigation revealed that EtxB induced only a minimal increase in proliferation above that of EtxB(G33D), in mixed cell cultures, and failed to induce any cell division of purified B cells or T cells. In contrast, receptor-binding by EtxB markedly up-regulated the expression of major histocompatability complex (MHC) class II, B7, intracellular adhesion molecule-1 (ICAM-1), CD40 and CD25 on the B-cell surface. These results indicate that the polyclonal effects of EtxB on B cells are not associated with wide-scale proliferation, but more likely with maintenance of B-cell survival by activation of molecules essential for B-cell differentiation.